

**CHARACTERIZATION OF RESPONSES OF *BRASSICA*
CULTIVARS TO ELEVATED CO₂ UNDER MOISTURE
STRESS CONDITION**

RANJAN DAS



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**DIVISION OF PLANT PHYSIOLOGY
INDIAN AGRICULTURAL RESEARCH INSTITUTE
NEW DELHI-110012**



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STRESS CONDITION**

BY

RANJAN DAS

**Submitted to the Faculty of Post-Graduate School,
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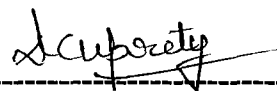
**FOR THE AWARD OF THE DEGREE
OF
DOCTOR OF PHILOSOPHY
IN
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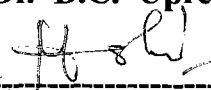
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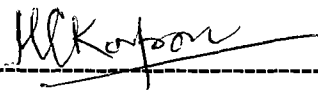
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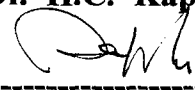
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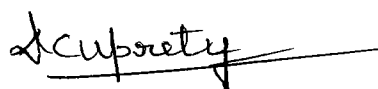
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CERTIFICATE

This is to certify that the thesis entitled “**Characterization of the responses of *Brassica* cultivars to the elevated CO₂ under moisture stress condition**”, submitted to the Faculty of Post Graduate School, Indian Agricultural Research institute, New Delhi, in partial fulfilment of the requirement for the award of the degree of **DOCTOR OF PHILOSOPHY in PLANT PHYSIOLOGY**, is a record of bona fide research work carried out by **MR. RANJAN DAS**, under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma. All the help and assistance received during the course of thesis investigation have been duly acknowledged.

New Delhi
November, 2003


(D.C. UPRETY)
Chairman
Advisory committee

DEDICATION

*- TO PADMA DAS
MY MOTHER*

*- IN THE FOND MEMORY OF
LATE RAJANI KANTA DAS
My Father*

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IARI, New Delhi

Dated: 22 November, 2003

Ranjan
(RANJAN DAS)

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1. INTRODUCTION

The carbon dioxide concentration in the atmosphere was in a steady state at $280 \mu\text{mol mol}^{-1}$ till the pre-industrial period (1850)(Table1). Measurements of CO_2 in the atmosphere, which began in Antarctica in 1957 and Mauna Loa (Hawaii) in 1958 indicated that its concentration is rising rapidly (Keeling, 1989). Mauna Loa studies also demonstrated that there was an increase of $1.0 \mu\text{mol mol}^{-1} \text{CO}_2$ per year from 1958 to 1982. The 1958 value of atmospheric CO_2 was $316 \mu\text{mol mol}^{-1}$. It is currently increasing at the rate of $1.5\text{-}1.8 \mu\text{mol mol}^{-1}$ per year (Houghton *et al.*, 1990)(Fig.1). This exponential rise of CO_2 in the atmosphere if continued at the present rate, it may be doubled by the end of 21st century (Luo and Moony, 1994).

Table 1. Atmospheric concentration of CO_2

Time	Concentration ($\mu\text{mol mol}^{-1}$)
Some 20,000 years ago	205
Prior to 1860 (past 1000 years)	274-280
Prior to 1900	290
1958	316
1982	340
1990	350
1995	365
1997	371
2000	373
2002	375

Measurements in New Delhi region also showed similar trend (Upreti 2000) (Fig. 2). The current rise in the concentration of CO_2 is due to the burning

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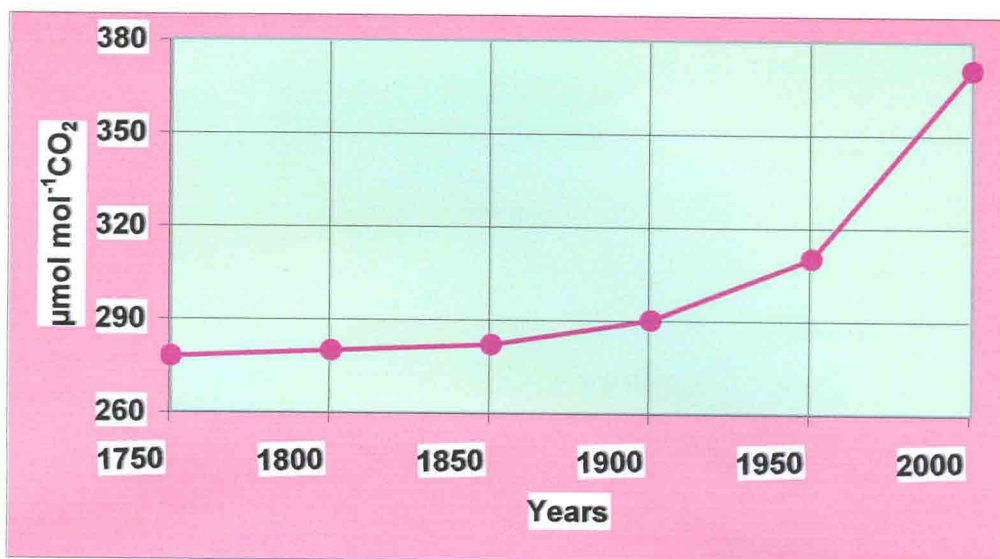


Fig. 1. Exponential rise of atmospheric CO_2 concentration (Houghton *et al.*, (1990); curve modified by Uprety *et al.*, 2000)

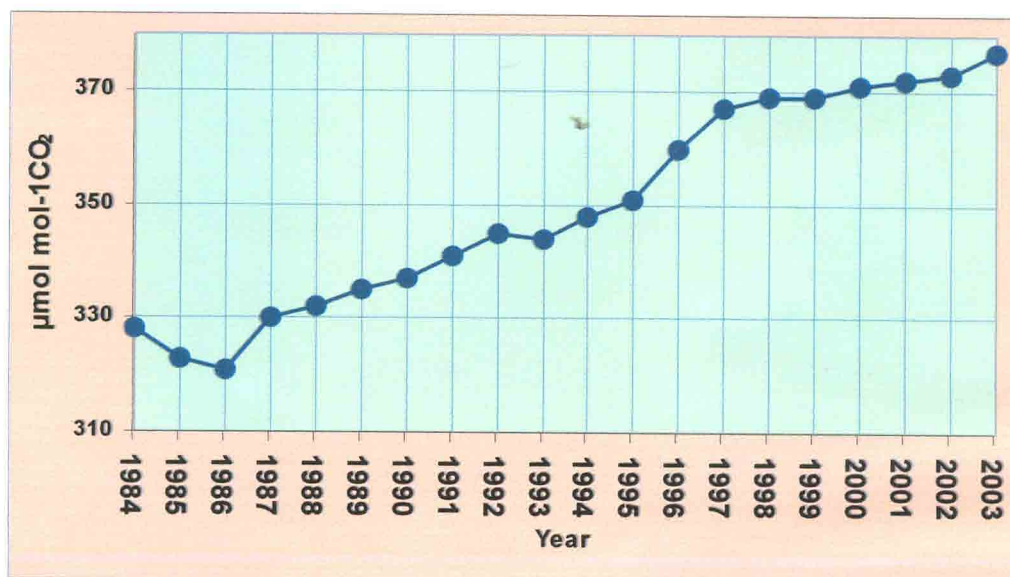


Fig. 2. The rise in atmospheric CO_2 , at IARI, experimental area (New Delhi) from 1984 to 2003 during winter crop season (Uprety *et al.*, 2003)

of fossil fuels, expansion of agriculture, urbanization and deforestation. This anthropogenic emission is adding about 8.0 GT of CO₂ per year in the atmosphere: 5.7 GT by fossil fuel burning and 2.3 GT by deforestation (Tans *et al.*, 1999).

The exponential increase of CO₂ in the atmosphere is an important climate change, which effectively influences the productivity of crop plants (Rosenzweig and Hillel, 2000). This rise in CO₂ concentration is expected to cause increase in the temperature of the earth by absorbing long wave heat radiations affecting cloudiness and precipitation (**climatic effect**). CO₂ has **biological effect** as well, because it serves as the main substrate for photosynthetic carbon assimilation. There is concomitant decline in photorespiratory activity and alteration in stomatal aperture (Long, 1991). It was reported that C₃ plants (Wheat, rice, oilseeds, pulses etc.) respond to elevated CO₂ since they reduce oxygenase activity of RuBP carboxylase oxygenase enzyme suppressing the photo respiratory activity. C₄ plants (Sorghum, Maize, Sugarcane etc.) show little or no photosynthetic response to elevated CO₂ because they are completely CO₂ saturated and not competitively inhibited by O₂ (Cure *et al.*, 1986).

However, there is no consensus on the quantitative effects of increased CO₂ on plant processes and growth, due to differences in response with stages of growth, type of species and crops and because of growth limiting environmental factors. The ability of farmers to survive/exploit these effects will depend on scientists accurately predicting growth and development responses of crops to such increase in atmospheric CO₂. Thus the selection of genotypes, which are likely to be more productive under the changed climatic condition, is a major imperative of global change research.

Innovative approaches for conducting long-term experiments have been developed to investigate the impact of rising atmospheric CO₂ on crops. Such studies are required as our crop cultivars were selected for the current CO₂ concentration and their response to the doubling CO₂ needs to be characterized

to develop the plant type for future. Earlier studies on plant responses to elevated CO₂ were conducted in closed chambers using short-term exposure in crops of temperate climate. However, studies were required on crops of **tropical/subtropical climate**, to generate database for their response to doubling CO₂ concentration under near natural condition. Such data, based on morpho-physiological and biochemical studies to characterize the responses of *Brassica* cultivars, have been collected in the present study to understand the strategies developed by the plants to adjust under changed environmental condition. This study may help for tailoring plant species for future CO₂ condition and determining nutrient management practice for their cultivation. It will also help to construct model to predict growth and productivity, so that farmers could be able to utilize and exploit this information to improve the productivity of *Brassica* species for future.

Several systems were used to study the response of plant community to elevated CO₂ (Allen, 1992). There were expensive, precisely controlled closed systems that continuously recondition and re-circulate the air in controlled environment of growth cabinets, phytotron, growth chamber etc. These systems, based on single component approach were highly effective way of discovering how one atmospheric factor influences crops, however, in practice several interactions with environmental condition will affect the responses. Though the cost and difficulties of studying CO₂ effects in the field were considerable, but such research needs to be done to give confidence arising from the simpler controlled environmental studies. There have been new approaches to CO₂ enrichment technology under field condition, which have potentially reduced the cost. Further, the computer models, which make prediction for the field situation, should at least partially be based on the data collected at the field level (Lawlor and Mitchel, 1991).

The current increase in atmospheric CO₂ at the rate of 1.5-1.8 $\mu\text{mol mol}^{-1}$ per annum is much too small signal for field experiment to detect a response. Hence, step CO₂ experiments using Open Top Chambers (OTCs) and Free Air

CO₂ Enrichment (FACE) technologies provide a large input signal or pulse that allows to trace crop responses for a short time frame. Currently most of the CO₂ experiments utilizing FACE and OTC facilities follow a holistic approach to design experiments in agro-ecosystem as natural as possible and then to observe their response.

Present investigation was done on *Brassica*, an important oilseed crop of Northwestern India. Among the oilseed crops, *Brassica* is the second important oilseed after groundnut contributing 30 percent of total oil production in India. Although India ranks first in terms of acreage, the production is low. The main reason for the low productivity is its large-scale rainfed cultivation (76%) (Chopra and Syam Prakash, 1991). Its cultivation experiences intermittent moisture stress at different stages of growth (Upreti *et al.*, 1995, Kumar and Yadava, 2001).

Earlier studies have shown the impact of climate change on this crop at different environmental conditions (Andreeva *et al.*, 1989). It was observed that elevated CO₂ was highly significant in mitigating the adverse stress effect on plant processes in *Brassica* species (Upreti *et al.*, 1995; Upreti *et al.*, 2003). It was also demonstrated that the CO₂ responsive characters could be transferred from the parent *B. campestris* to the hybrid *B. oxycamp* (Upreti *et al.*, 1998).

In the present investigation, an attempt has been made to analyze the interactive effect of elevated CO₂ and moisture stress on *Brassica species*. This study was done to understand the differential response of *Brassica juncea* cv. 'RH-30' and *Brassica campestris* cv. 'Pusa Gold' to the interaction of elevated CO₂ and moisture stress on various parameters of plant physiological processes, morpho-physiological attributes of growth and development comprising of water relations and oxidative stress regulatory system, anatomical adaptations and grain quality components. This will help in characterization of the responses of *Brassica* species to the interaction of elevated CO₂ and moisture stress. Such characterization will be the basis in determining the cultivation and nutrient management for these cultivars to suit higher CO₂ atmospheric condition.

2. REVIEW OF LITERATURE

Global Climate Changes

The accelerating changes in the earth's environment are being driven due to the growth in human population by the increasing level of resource consumption, changes in the technologies and socio-political organizations. Global climate changes, which affect the agriculture, are:

1. Changes in land use and land cover
2. World wide decline of biodiversity
3. Changes in atmospheric composition of gases and
4. Changes in climate

One of the major driving forces for global climate change is the rapid increase in the greenhouse gas content in the atmosphere. Per capita CO₂ emission from south Asia was 0.47 Tg (1995) whereas its projected value is 0.90 Tg for 2050. The total regional emission of GHG (in CO₂ equivalents) is 3 per cent of the global total. Its annual growth rate is as high as 6 per cent for India, 8 per cent Bangladesh and 10 per cent Pakistan (IPCC, 2001).

Agricultural emission is 0.2 TC (tons of carbon) per person for India, 1.2 TC global average per person and 5.0 TC per person for USA. The global atmospheric CO₂ was ranging between 274-280 $\mu\text{mol mol}^{-1}$ in a steady state during the pre-industrial period. It has been rising since then and is currently about 1.8- $\mu\text{mol mol}^{-1}$ per annum (Houghton *et al.*, 1990), accumulating about 8.0 GT C per year with atmospheric level increasing by about 0.5 per cent per year. The use of fossil fuel puts about 5.7 GT per annum CO₂ whereas deforestation adds 2-

3 GT per annum. Of this about 3.3 GT per annum remains in the atmosphere accounting for the rise in atmospheric concentration and remainder being absorbed by oceanic and terrestrial biota (Tans *et al.*, 1999). The land biosphere appears to have gained carbon immediately after deglaciation and then leaked 10 per cent of its carbon content i.e. about 200 GT C. The ocean was largest recipient and gained 150 GT carbon, whereas, atmosphere retained 50 GT C (Ciais, 1995). An estimated 590-1180 million tones of carbon was locked up in cropland soil alone in the form of soil organic matter from crop residues and manure in 1997-99. Mean sea level was projected to rise by 15-20 cm by 2030 and 50 cm by 2100. If the present emission pattern continues in the future, atmospheric CO₂ will be doubled by end of the 21st century.

Average global temperature was projected to rise between 1.4 and 5.8 °C by 2100 and by 2030 the increase will be lesser (FAO, 2002 synthesis report). The human activity may be making the greenhouse gas blanket “thicker”. For example burning coal, oil, and natural gas spew huge amounts of carbon dioxide release in to air; the destruction of forest allows carbon stored in the trees to escape into atmosphere and other activities such as raising cattle and planting rice emit methane, nitrous oxide, and other greenhouse gases (Hansen, 1988 and Fuller, 1997) (Fig 3). It has been scientifically proved that increase in the concentration of these gases has led to an average, additional warming of the earth’s surface and atmosphere, threatening to change the climate and impact the entire earth system in an unpredicted manner. The rise in CO₂ in atmospheric has been documented continuously since 1958 by Keeling *et al.* 1989. After Mauna Loa studies, Keeling’s (1989) reported that the rise of CO₂ showed a saw tooth pattern model reflecting the seasonal cycle of vegetation growth (Fig. 4). India contributes only 2 per cent of the total CO₂ emission of world, where as highest contribution recorded in USA followed by Germany (WB, 2001, Fig. 5). As CO₂ contributes major share for global warming (almost 50%) its rise in the atmosphere is likely to affect global climate and to cause regional changes in air temperature and humidity, length of

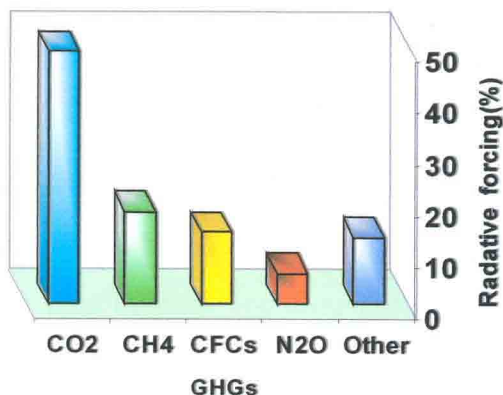


Fig. 3 Contribution of green house gases to Global warming [Hansen *et al.*, 1988]

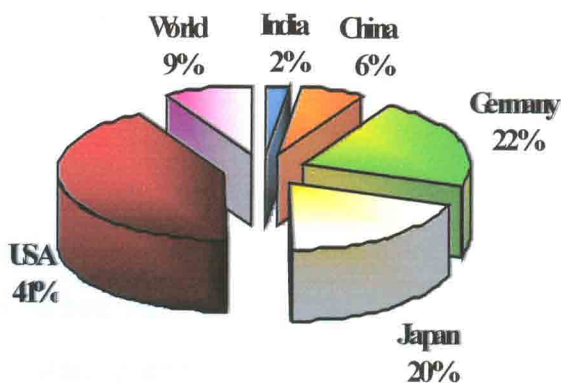


Fig. 5 Percent contribution of CO₂ emission from different countries [Source: WB, 2001]

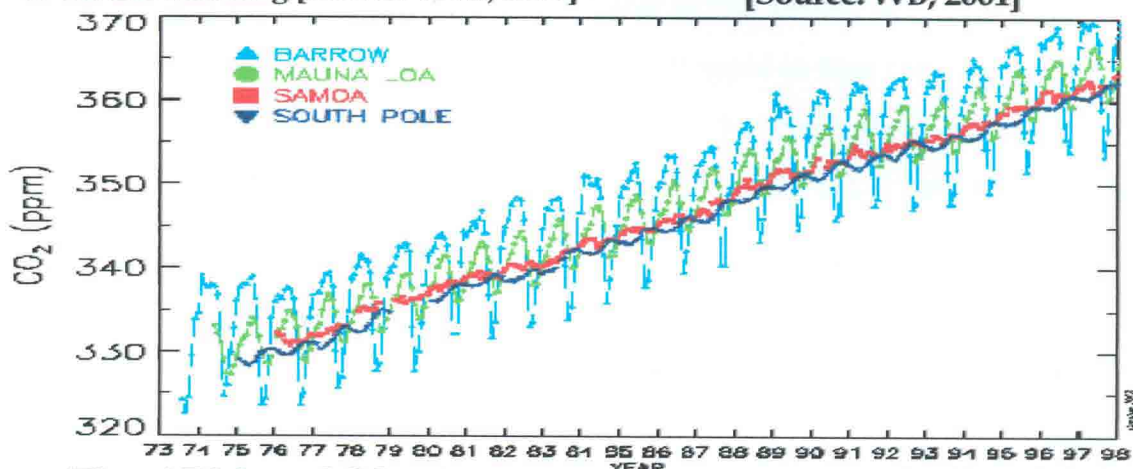


Fig. 4 Rising of CO₂ showed a saw tooth pattern [Keeling, 1989]

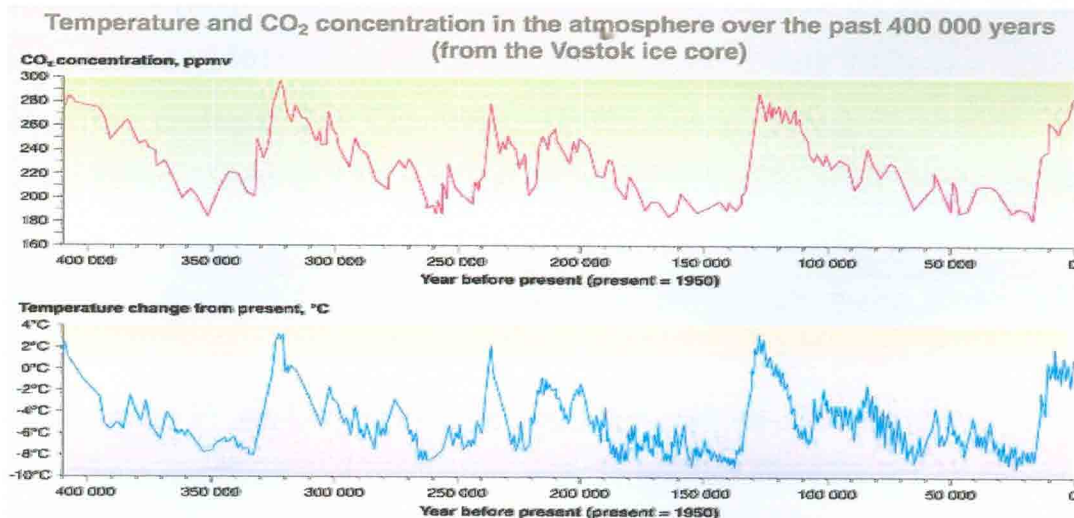


Fig. 6 Vostok ice-core data

Source: J.R. Petit, J. Jouzel, et al. Climate and atmospheric history of the past 400 000 years from the Vostok ice core in Antarctica, *Nature* 399 (400), pp 429-436, 1999.

growing season, precipitation and evaporation, all of which will effect plant water relations (Jarvis, 1989).

There is legitimate concern over how the predicted doubling of the CO₂ concentration during the next century will influence the earth's climate, ecosystems, and agricultural production; but substantial fluctuations in CO₂ have occurred in the past (Vostok ice core data) (Fig. 6). When plants made the transition to land, some 420 million years ago (MYA), the atmosphere may have contained as much as 4000-6000 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ (Boger, 1980). The rise of vascular plants, and their attendant photosynthesis, was a major factor in the decline of the atmospheric CO₂ concentration, which by 300 MYA may have dropped to near present values (Bowes, 1996). Thus the atmospheric CO₂ concentration has fluctuated perhaps by as much as 20-fold over geological time; so higher values are not a new phenomenon for plants (Bowes, 1996).

History of CO₂ and crop response study

Record of research on plant responses to CO₂ has been shown as early as 1804 when de Sassure noted the faster growth in pea plant with an elevated CO₂ atmosphere (8%). The first description of the CO₂ green house effect was reported Jhon Tindal (1861). Swedish Nobel prizewinner Svante Arrhenius (1896) and American geologist T.C. Chamberlin (1899) first published an account on green house effect. However, CO₂ fertilization was practiced by green house grower over the last 200 years using the decomposition of organic material as a source of CO₂. By 1861, about 4000 acres of green house crops were being grown with CO₂ enrichment in the Netherlands. The first CO₂ experiment in the field was carried out in 1920-23 by Lundegardh on sugarbeat and oat. He observed 16 per cent increase sugarbeat root production with 15 per cent increase in CO₂ concentration (from 284-328 $\mu\text{mol mol}^{-1}$) whereas oat production was 30 per cent more for doubling of CO₂ concentration from 282-594 $\mu\text{mol mol}^{-1}$. Kimball (1983) reviewed 430 observations and reported 33±6 per cent increase in average yield of various crops due to doubling of CO₂ above ambient level (Table 2).

The response of plants to the elevated CO_2 has been documented in numerous paper and review articles ranging from detailed biochemical to broad eco-physiological studies (Johnson *et al.*, 1993). It must however, be emphasized that most of the findings on physiological and allocation response to CO_2 were first discovered in agricultural crops.

Govt. of India and Indian Council of Agricultural Research started impact assessment analysis of crop responses to rising atmospheric CO_2 in Indian Agricultural Research Institute through a National Fellow Project (1995) and cress fund project (1993) with following objectives:

- (A) To develop facilities of CO_2 enrichment technology
- (B) Study the responses of crop plant to elevated CO_2
- (C) Characterize the responses of crops to tailor plant for higher CO_2 environment and
- (D) Identifying the crop cultivation and nutrient management technology for them.
- (E) Application of crops response data in preparing the model and developing plant types for further CO_2 enrichment (Uprety, 1993)

Table 2. Historical observations on CO_2 enrichment in plants (Modified version of Wittwer, 1986) in CHRONOLOGICAL ORDER

Year	Observations	Observer
1648	Major increase in mass of a willow came from the atmosphere	Van Helmont
1804	First observations of CO_2 enrichment on plant growth	de Sassure
1894-1902	Positive effects of CO_2 enrichment on plant growth	Demoussy
1902	Negative effects of CO_2 enhancement on plant growth	Brown & Escombe
1918	Positive effects of CO_2 on plant growth (U.S.A.)	Cummings & Jones
1931	600 nurseries reported using CO_2 in Germany	Reinau

1959	Basic studies on CO ₂ and light responses in plants	Gaastra
1961	Dutch growers add CO ₂ for improving yields of 400 acres lettuce	Anon
1962	Response of cucumber reported to the complimentary effects of CO ₂ and light	Hopes & Ries
1962-1966	Response of flower crops reported	Goldberry & Holley
1964	Comprehensive studies on tomato & cucumber	Wittwer & Robh
1976	Positive effects noted for the growth of tree seedlings	Hannover <i>et al.</i>
1987	Reduction in transpirational cooling in leaves of cotton	Idso <i>et al.</i>
1983-1986	Observed 31 per cent increase in agricultural plant productivity	Kimball
1983-1987	64 per cent increase in cotton yield both (lint+seed at 650 l/L CO ₂)	Kimball <i>et al.</i>
1995-2003	Elevated CO ₂ ameliorated the adverse moisture stress effect in <i>Brassica</i> plants. Demonstrated the possibilities of transferring the CO ₂ responsive characters from the parent <i>B. campestris</i> to the hybrid <i>B. oxycamp.</i> Study of response of rice cultivars to elevated CO ₂ .	Uprety <i>et al.</i>

Biological effect

Plants have been directly affected by rising atmospheric CO₂ concentration because CO₂ is the first molecular link from atmosphere to biosphere. Being the primary raw material for photosynthesis, CO₂ becomes the nerve of the entire food chain (Roger *et al.*, 1994) and is the basis of almost all life on the earth (Idso, 1995). Though most of the CO₂ research have been conducted on monoculture of agricultural species, it is possible that the complexion of natural plant stands with their numerous competing species will change even more in the future higher CO₂ world than will cultivated fields with only a few domesticated species (Kimball, 1995). The natural vegetation is also expected to show significant

response to rising CO₂, since 95 per cent of the species of higher plants and 100 per cent of the trees are C₃ plants (Drake *et al.*, 1992).

The higher level of atmospheric CO₂ is likely to affect C₃, C₄, and CAM plants differentially (Poorter, 1993). Atmospheric CO₂ enrichment typically enhances the growth rates of almost all plant (Idso, 1999 and Kimball, 1983) but enhancement is very significant in C₃ species rather than C₄ (Poorter, 1993; Idso *et al.*, 2000 and Kimball *et al.*, 2002). C₄ system represented an evolutionary adaptation to low CO₂ environments (Hall and Allen, 1993) and has lower CO₂ compensation point. With the engineering of the plants with higher production potentials, the ambient CO₂ on agriculture is apparent from the fact that out of the 20 most important food crops in the world, 16 have the C₃ photosynthetic pathway, while out of the world's 18 most abnoxious weed, 14 have the C₄ photosynthetic pathway. Interestingly, the first detected response to the increasing CO₂ was the increase in growth rate of bristlecone pine trees observed one and a half century ago (La Marche *et al.*, 1994)

Plants with C₄ photosynthetic pathway have negligible photosynthetic response to elevated CO₂ because the C₄ cycle increase the CO₂ concentration in bundle sheath cells to the point where very little photorespiration occurs and the Calvin cycle is nearly saturated with CO₂ (Conroy, 1992, Ziska *et al.*, 1999). Response of C₄ plant to elevated CO₂ depends upon the abiotic factors because recent study has shown that free air CO₂ enrichment enhances C₄ photosynthesis in *Sorghum bicolor* in the field and increases crop yield and biomass production during drought condition (Cousins *et al.*, 2001; Ottman *et al.*, 2001 and Wall *et al.*, 2001). CO₂ influenced the process of photosynthesis in some of the plants by reducing the photorespiratory activity due to its influence on the oxygenase fraction of RuBP carboxylase, the key enzyme of C₃ photosynthetic pathway. However, C₄ plants showed little response to elevated CO₂ as its saturation already existed. C₃ plants do not posses CO₂ saturation system and the first enzyme in photoreductive C cycle (Rubisco) operates well bellow its K_m due to competitive

inhibition by oxygen, hence, show significantly positive response to higher CO₂ concentration.

Crop

Oilseed crops play a vital role in India's agricultural economy. These crops hold a sizeable share of 13 per cent of the country's gross cultivated area, contributing around 5 per cent of its gross national production and about 10 per cent of the value of agricultural products (Anonymous, 1997). Among oilseed crops, rapeseed and mustard group is the second most important after groundnut contributing nearly 30 per cent of the total oil production in India. They play an important role in agriculture, industries and foreign trade. India ranks first in acreage of mustard in the world occupying an area of 5.83 million hectares of land and a total production of 5.34 million tones with an average yield of 1106 kg/ha (Chakravarty and Gautam, 2002). The annual requirement of oilseeds in India is much higher than the production and to bridge the gap, government is spending huge amount of foreign exchange in importing oilseeds. Therefore, there is an urgent need to increase oilseed production in this country when the requirement will be double that of the previous production. Government of India appointed a Technology Mission on Oilseed (TMO) in 1986 to harness the best production, processing and management technologies to accelerate the self-sufficiency in oil seed and vegetables oils. This has brought major change in oilseed production (Anonymous, 1997).

Mustard showed remarkable progress in terms of production and productivity that touched a peak of 6.66 m tones and 1017 kg/ha respectively during 1996-97. The production scenario of oilseeds in India can be broadly categorized into three distinct phases i.e. before 80s during which the oilseeds production rose slowly and steadily, 80s and early 90s in which the increase in oilseed production was very sharp and the current phase is characterized by stagnation in the oil seed production and productivity (Kumar and Yadava, 2001).

Table 3. Area, production and yield of rapeseed mustard (Prepared from Survey of Indian, Agriculture 2002 and FAS, USDA 2003)

Year	Area (mha)	Production	Yield (kg/ha)
1994-1995	6.01	5.76	958
1995-1996	6.55	6.00	916
1996-1997	6.55	6.66	1017
1997-1998	7.44	4.70	668
1998-1999	6.00	5.77	875
1999-2000	6.32	6.12	968
2000-2001	5.25	4.50	860
2001-2002	4.08	3.60	750

The reasons for low productivity includes

1. The large scale rainfed cultivation (76% of *Brassica* grown area is rainfed),
2. Minimal resource capacity of the small and marginal farmers,
3. Comparatively low technology of production for this crop, and
4. Vulnerability of this crop to pests and diseases.

Brassica is an important oil seed of Northwestern India. This crop experiences intermittent drought during its growth period from vegetative to the siliqua formation stage and their variability in the responses of these cultivars to the moisture stress (Chopra and Shyam Prakash, 1991 and Labana *et al.*, 1992). The *B. campestris* was susceptible to drought compared to *B. juncea* (Upreti *et al.*, 1995). The consumptive use of water in rapeseed/mustard varies from 200-300 mm. The cultivation of rapeseed mustard in Indian sub-continent is mainly confined to the Indo-Gangetic and Brahmaputra plains and sub-mountainous regions (Fig.7).

CO₂ enrichment technology

Most of the research on plant responses to high CO₂ concentration have

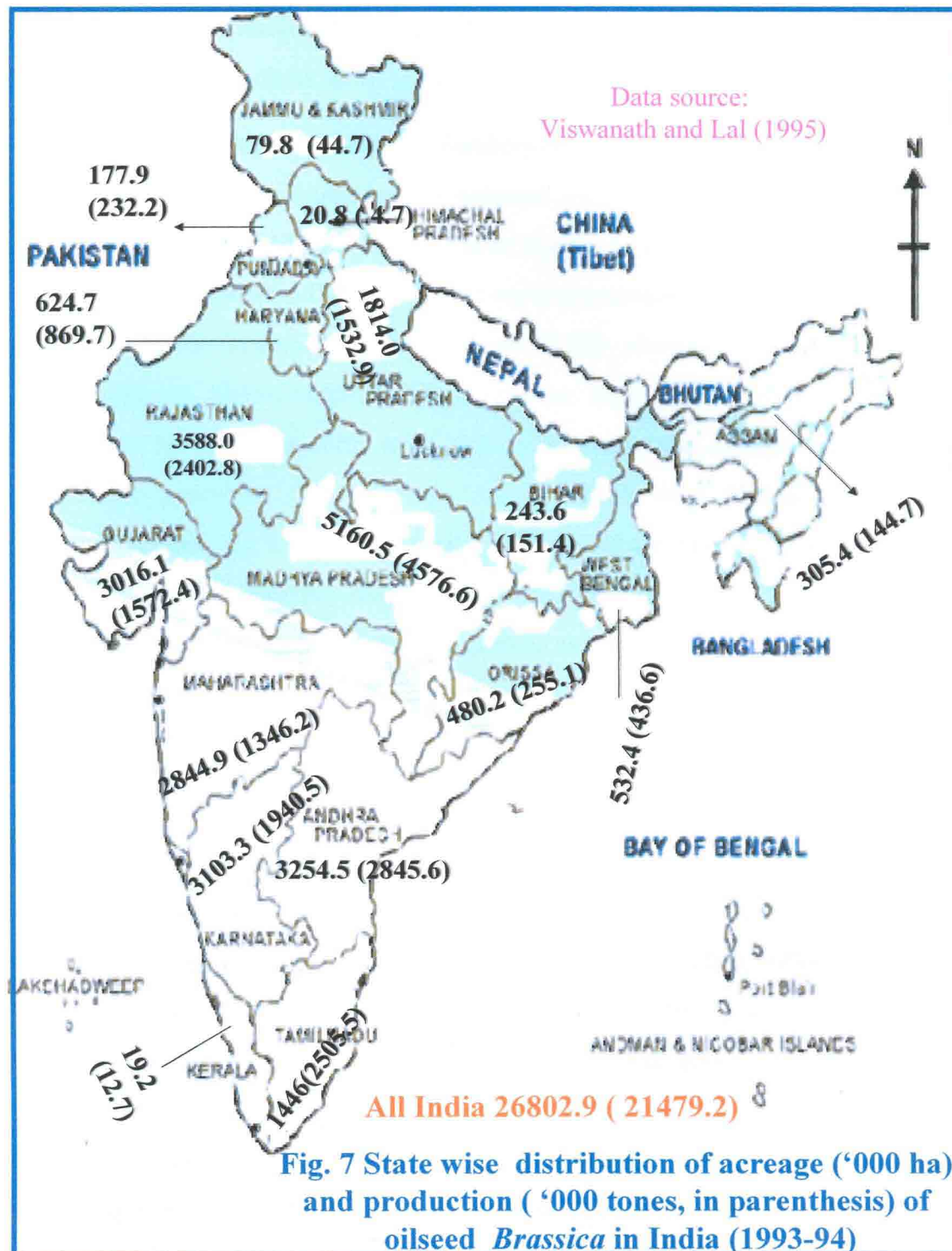


Fig. 7 State wise distribution of acreage ('000 ha) and production ('000 tones, in parenthesis) of oilseed *Brassica* in India (1993-94)

been conducted in the past under laboratory, greenhouse or controlled field condition (Bazzaz and Garbutt, 1988). With the development of temperature control in green house, the studies on interactive effect of CO₂ and temperature on plants became possible (Mitchell *et al.*, 1995).

Sunlit controlled-environment chambers based on “closed-loop” air circulation systems with computer-managed environmental controls were developed in the late 1950s and early 1960s (Allen, 1992). These chambers usually had Mylar polyester film walls. Non-dispersive IRGAs allowed rapid measurement of CO₂ concentration, and these analyzers in combination with metered CO₂ allowed direct and continuous measurement of photosynthetic CO₂ exchange rate. These systems were the predecessors to the units with large controlled root-zone containers as well as canopy zone chambers (Phene *et al.*, 1978), which were further modified for improved systems for CO₂ effect studies. These SPAR chambers were designed to provide accurate, flexible control of dry-bulb temperature, CO₂ concentration and humidity of canopy air, as well as control and measurement of soil water and root conditions. The SPAR chamber system described by Jones *et al.* (1984b) provided measurement of canopy photosynthetic rate and transpiration rate at 5 min intervals. Air sample from each chamber were analyzed for CO₂ on a 5 min cycle.

Open-top field chambers as described by Heagle *et al.* (1973) have extensive use as plant exposure units both in air pollution and in CO₂ effects studies in the field. These systems have been used to expose potted plants, annual crops, and trees to a variety of aerial pollutants and CO₂, and they are currently in use at a number of laboratories in North America and Europe. Hardey and Havelka (1975) first used a square wall, open top enclosure to expose soybeans to atmospheres enriched with CO₂ for the purpose of studying the effect of increased photosynthate production on symbiotic nitrogen fixation. Rogers *et al.* (1983) adapted the basic cylindrical open top chamber system to generate large-scale CO₂ test atmospheres in the field. Nakayama and Kimball (1988) used a square-wall,

open-top chamber with 0.2 m diameter perforated polyethylene rows of cotton. Drake *et al.* (1989) developed an open-top chamber system for CO₂ enrichment of saltmarsh vegetation that re-circulated part of the input air. Computerized control and data acquisition systems have been developed (Nystrom *et al.*, 1988b).

Open top chamber (OTC) is one of the most widely used techniques for studying elevated CO₂ impact on crops and ecosystem. Climate in OTCs tracks the dynamic change in temperature, light, rainfall etc. experienced in unclosed area without costly and complex environmental controls characteristics of closed chambers (Jones *et al.*, 1984). CO₂ enrichment studies at Indian Agricultural Research Institute, New Delhi were done in open top chambers. These chambers measure dynamic changes in temperature, light and rainfall experienced by unclosed areas with inexpensive and complex environmental controls (Upreti *et al.*, 1995, 1998). OTC subjects the whole plant to the treatment without restricting root growth. The principal advantage of OTC is the ability to provide control over the atmospheric variations to which plant are exposed while maintaining more or less edaphic conditions of the field setting (Hendrey *et al.*, 1992).

Although CO₂ experiments in control environment have the advantage of easy CO₂ regulation, but the basic information generated there may differ significantly from the ambient condition (Lawlor, 1996). To overcome these limitations, the FACE system provided a unique opportunity to examine the effects of growth at elevated CO₂ and other under environmental conditions without any direct perturbation of microclimate (Hendrey and Kimball, 1994).

The need to study CO₂ effects on vegetation in a natural field environment has led to the concept of artificially elevating CO₂ by release through an array of pipes. The FACE approach had much in common with methods developed by air pollution ecologist. A grid release system to study air pollutant effects in vineyards for the French Ministry of Agriculture was described by deCormis *et al.* (1975). By using free air CO₂ enrichment (FACE) technology, the general microclimate of the area under study was unaltered and experimental research can

be conducted at the level of the ecosystem (Miglietta *et al.*, 1997 and Hendrey *et al.*, 1999).

New advanced Free Air CO₂ Enrichment (FACE) technique was also developed in India for south Asian region at Indian Agricultural Research Institute, New Delhi. It is simpler and cost effective design primarily based on good features of Italian and Japanese FACE. It has an eight-meter diameter ring of octagonal shape. Therefore it is called mid-FACE. The estimated CO₂ consumption in this system is approximately one tenth of Arizona FACE (Maini *et al.*, 2002). Multi-country and multidisciplinary experiment were being done in Indian Agricultural Research Institute, New Delhi using mid FACE (Upreti, 2003).

Recently, Petersen *et al.* (2001) described the design and performance of a miniature free air CO₂ enrichment (Mini-FACE) system. According to them, in this system two joined concentric rings (a CO₂ fumigation vent-ring and an outer baffle-ring which allows enhanced local mixing) can be adjusted vertically, allowing the fumigation system to trace canopy development during crop growth. These features facilitate the use of relatively small plots (1.14 m in diameter). In tests based on four rings, winter wheat was exposed to elevated CO₂. The system maintained a 1-minute average CO₂ at the center of the control ring within ± 10 per cent of the set point for 77 per cent of the operation time. In the vertical plane, where the CO₂ is liberated (level 0), the set point CO₂ ± 20 per cent was met for 77 per cent of the time given as a weighed mean of the ring area (1.02 m²). The variation in CO₂ measured among the four rings was acceptable. This flexible and cheap system may provide widespread research in the effects of increased atmospheric CO₂ on field-grown small-grain crops.

Plant growth, biomass production and yield

The growth responses of crops to the elevated CO₂ have been reviewed by Acock *et al.* (1986). They suggested that the order of priorities for the use of photo-assimilates by plants is (a) survival, (b) reproduction, (c) growth of existing organs, (d) increase in the number of existing organs (mostly by branching

or tillering), and (e) storage. When these needs are met, the plants may sustain CO₂ fixation in the face of an imbalance of the source/sink ratio for photo-assimilates. As with photosynthesis rate, growth and yield, plants show a wide range of responses to the increasing CO₂ (Allen *et al.*, 1994). Allen *et al.* (1987) used a nonlinear model to predict photosynthetic, final biomass, and seed yield responses of soybean to CO₂ relative to a CO₂ concentration of 330 $\mu\text{mol mol}^{-1}$.

Table 4. Soybean responses to rising CO₂ predicted by the nonlinear, hyperbolic, modified Michaelis-Menten model

Soybean responses Year	CO ₂ change	Photosynthesis ($\mu\text{mol mol}^{-1}\text{CO}_2$)	Biomass % increase	Seed % increase
1800- 1958	276-315	12	10	8.0
1800-1986	276-345	20	17	13.0
1958- 2058	315-630	53	43	32.0

Source : Ref. Allen *et al.* 1987

Table 4 shows that the rise of CO₂ from pre-industrial values to present values should cause a 13 per cent increased yield, furthermore, a doubling from 315 to 630 $\mu\text{mol mol}^{-1}$ could cause a 32 per cent increase in seed yield; an increase that is in close agreement with the survey of plant responses to a doubled CO₂ increase (Allen *et al.*, 1994). According to them the seed yield responses were less than biomass responses because of greater vegetative growth and less efficient conversion to seed under high levels of CO₂. Higher temperature in combination with CO₂ enrichment appears to enhance vegetative biomass accumulation in C₃ plants, and this relationship might contribute to a negative feedback on the atmospheric CO₂ increase (Woodwell *et al.*, 1995). Reproductive growth of plants appears to respond quite differently to temperature increases than vegetative growth (Baker *et al.*, 1990).

Working on 16 crop species, Morison and Gifford (1984a) reported that high CO₂ increased leaf area in all but two species, the increase varied from 20-

75 per cent. In oilseed rape (*B. napus* L.) the leaf area increase with high CO₂ was 40 per cent. Morison and Gifford (1984b) showed that plant dry weight was increased by high CO₂ in 16 out of 18 test species at the first harvest (average increase of 65 per cent, range from 26 to 132%). The increase in dry weight with high CO₂ (average of 53%) was associated with increase in WUE of between 40 and 80 per cent (average of 67%). The increase in dry weight was 83 and 48 per cent and WUE increase was 64 and 50 per cent and root to shoot ratio was 0.32 and 0.39, respectively with enriched CO₂ concentration at the initial stage and whole growth period in *Brassica napus*.

Cure *et al.* (1987) exposed young vegetative soybeans to control and enriched CO₂ environments continuously for 22 days and found increased whole plant dry weight. The initial dry weight increases were associated with the accumulation of non-structural assimilates in leaves and increased specific leaf weight. The total number of leaves, length of stem, total leaf area, overall growth rate, total leaf, stem and root dry weight responded positively to increased concentration CO₂ in poplar clones (Radoglou and Jarvis, 1990).

Baker *et al.* (1990) reported that in rice (cv. IR-30) total shoot biomass, root biomass and tillering were increased. The greatest increase occurred at 500 $\mu\text{mol mol}^{-1}$ CO₂ treatments. Bunce (1990) recorded that biomass of soybean and tomato plants was greater at the higher CO₂ but not in a C₄ species, *A. hypochondriacus*. Bunce and Caulfield (1991) in a study, revealed that elevated CO₂ increased the rate of biomass accumulation in all the three test species *D. glomerate*, *L. perenne* and *M. sativa* as compared to the ambient CO₂. Winter wheat grown with ample nutrition and water in 700 $\mu\text{mol mol}^{-1}$ CO₂ under simulated conditions of Rothamsted has 15-40 per cent greater biomass (Lawlor *et al.*, 1993 and Mitchell *et al.*, 1993).

Mitchell *et al.* (1996) grew winter wheat in a controlled environment under field condition at 360 $\mu\text{mol mol}^{-1}$ and 690 $\mu\text{mol mol}^{-1}$, where some plants were shaded to mimic cloudy condition for 20 days between terminal spikelet and start

of grain fill. They found that the elevated CO₂ increased grain yield by about 20 per cent while shading in any period decreased yield and no interaction between these effects were significant for grain yield, though there were complex interactions for mean grain size. Ziska *et al.* (1996) subjected to 17 contrasting rice cultivars to 373 and 664 $\mu\text{mol mol}^{-1}$ CO₂ concentrations. They observed that growth at high CO₂ increased by an average of 70 per cent and 22 per cent respectively for growth temperatures of 29/21 and 37/29 °C for all cultivars. At 29/21 °C, a 50 per cent increase in average grain yield was obtained with enriched CO₂.

Tognetti and Johnson (1999) reported that elevated CO₂ increased biomass production only in the high-N treatment in *Quercus ilex* L. Fine root/foilage mass ratio decreased with high-N treatment and increased with CO₂ enrichment. There was evidence of a preferential shift of biomass to belowground tissue at a low level of nutrient addition.

Segura *et al.* (2001) studied the effects of CO₂ enrichment on the bio-productivity and nutrition of a cucumber (cv. *Marumba*) under greenhouse conditions and they found that the treatment with CO₂ increased total accumulated dry matter by 14.4 and 13.3 per cent in the first and second crop cycles, respectively. The nutrient content in aerial organs, expressed in per cent of dry matter, was also affected by CO₂ enrichment. The crop grown in a CO₂-enriched atmosphere consumed a greater amount of nutrients, mainly N, K, Ca and Mg.

Liu *et al.* (2002) reported that elevated CO₂ resulted in larger fresh and dry mass and higher root-to-shoot ratio in two-year-old needles in young trees of Sitka spruce (*Picea sitchensis*). Suter *et al.* (2002) reported that grass swards showed a higher root-shoot ratio of dry matter (R: SDM) at elevated CO₂ than at ambient CO₂. Elevated CO₂ in the field increased root dry matter (109%), R : SDM (44%) and, in accordance, root-shoot ¹⁴C ratios (R : S¹⁴C, 39%).

Atmospheric carbon dioxide is known to increase the plant yield. Kimball (1983) reviewed 430 observations of carbon dioxide-enrichment studies conducted prior to 1982 and reported an average yield increase of 33±6 per cent for a doubling

of the carbon dioxide concentration. This value has been generally confirmed by many other studies since that time. The yield increases seem to apply for both biomass accumulation and grain yield. Thus, plants may grow larger and use more water as the global carbon dioxide concentration increases. Soybean seed yields and biomass yields are predicted to increase 31 and 41 per cent respectively, from a doubling of carbon dioxide.

Downton *et al.* (1987) studied the response of elevated CO₂ (800 µmol mol⁻¹) on 3-year-old fruiting Valencia orange (*Citrus sinensis*) scions on citrange rootstock over a 12-month period under controlled environment. They recorded a 5.8 per cent increase in fruit yield at higher CO₂. Baker *et al.* (1990) reported doubling of CO₂ resulted in a 32 per cent increase in grain yield in rice (cv. IR-30). Nijs *et al.* (1988) observed that canopies of white clover grown at high CO₂ levels showed a 75 per cent average increase in productivity. Mitchell *et al.* (1993) reported that winter wheat when grown in elevated CO₂ (700 µmol mol⁻¹), grain yield was increased 18-23 per cent compared to control. Segura *et al.* (2001) in his studies reported that fruit yield increase by 18.4 per cent in cucumber (cv. *Marumba*) when grown in elevated CO₂.

Seed quality

Much less, however, is known about the effects of elevated concentration of atmospheric CO₂ on the nutritive value of food (Lawlor and Mitchell, 1991). Frick *et al.* (1994) studied the effect of N level under controlled-environment conditions i.e., with ambient or increased CO₂ concentration (998±12 µmol/mol). According to him seed oil content in *Brassica* was not significantly affected by CO₂ enrichment. Seed oil content was accompanied by a decrease in seed protein. The reduced transpiration could also be responsible for reduction in nitrogen uptake resulting in lower protein content in harvested *Brassica* grain as reported by Uprety *et al.* (1995). Decline in wheat ear N or grain N was detected by Hocking and Meyer (1991) and Conroy (1992).

According to Uprety *et al.* (1997) elevated CO₂ brought about a significant increase in carbohydrate component in the *Brassica* seed. The increase in starch content (37.5%) followed by reducing (28%), total (24%) and non-reducing sugars (22%). The elevated CO₂ significantly reduced protein content (10.3%) in the seeds. CO₂ enrichment also increased the oil content (10.4%). The accumulation of carbohydrates may possibly lead to reduction in protein content due to dilution effect (Wong 1990). Field *et al.* (1992) indicated that elevated CO₂ tended to increase carbohydrate reserve and plant biomass thus diluting N for the plant and reducing N concentration. This higher carbohydrate status as associated with a high C : N ratio.

Akin *et al.* (1995) reported that elevated CO₂ concentration increase the content of acid detergent fibre and cellulose of young leaves of wheat results increased digestibility. They also reported that under elevated CO₂ acid detergent lignin reduced along with protein.

Heagle *et al.* (1998) studied the interactive effects of O₃ and CO₂ on yield and seed quality of soybeans grown in open-top field chambers. Plants were exposed from emergence to maturity to four CO₂ levels (ambient and 1.3, 1.6 and 2.0 times ambient) and three O₃ levels (0.4, 0.9 and 1.5 times ambient) in 12 combinations. Increasing O₃ suppressed growth and yield, whereas CO₂ enrichment stimulated growth and yield. Carbon dioxide-induced stimulation was greater for plants stressed by O₃ than for non-stressed plants. Effects of O₃ and CO₂ on seed oil content were variable, with numerous cultivar differences. Seed protein content was never affected. Elevated O₃ suppressed oleic acid content in seeds, whereas CO₂ increased it; the nature of the O₃ x CO₂ interaction for oleic acid was similar to that observed for most yield measures.

Idso *et al.* (2000) study of various anticancer and antiviral agents in lily plant under elevated condition. He reported that these agents were increased 7-8 per cent under elevated CO₂.

Idso *et al.* (2002) reported that elevated CO₂ not only increase fruit production but also increase the vitamin C concentration by 7 per cent in juice of sour orange.

Growth studies

Growth analysis techniques were used by number of scientists to assess the interactive effect of CO₂ and water stress on growth and biomass partitioning in different crops.

Jolliffe and Ehret (1985) reported that CO₂ enrichment caused large and rapid increases in leaf dry weight, unit leaf rate and SLW in bean leaves. Cure *et al.* (1987) observed increased in whole plant dry weight of soybean under elevated CO₂. The increase in initial dry weight was associated with the accumulation of nonstructural assimilate in leaves and increased specific leaf weight (SLW).

Campbell *et al.* (1988) found that soybean (cv. 'Bragg') grown at elevated CO₂ had a greater SLW and 44 per cent heavier pod weight. Higher levels of CO₂ tend to decrease SLA due to thick cell walls and greater C content (Clough and Peet, 1988). Hoddinott and Jolliffe (1988) in their investigation with *P. vulgaris* at 380, 800 and 1400 ppm observed that elevated CO₂ increased the SLW significantly, indicating a denser leaf structure. Vu *et al.* (1989) observed that soybean grown under 800 $\mu\text{L L}^{-1}$ CO₂ showed 37 per cent increase in SLW over control plats at 330 $\mu\text{L L}^{-1}$ CO₂.

Yelle *et al.* (1990) observed that the elevated CO₂ concentration increase RGR of *L. esculentum* and *L. chmielewskii* by 18 and 30 per cent respectively after two weeks treatment. The NAR and SLW were always higher in CO₂ enriched plants, suggesting that assimilate were preferentially accumulated in the leaves as reserves rather than contributing to leaf expansion.

Retuerto and Woodward (1993) reported that CO₂ enrichment significantly increase the rate of biomass accumulation and the relative ratio of biomass increase to leaf area expansion in *Sinapis alba* so LAR and SLA under elevated significantly

reduced compared to ambient. This reduction also may be due to an increase in the bulk density of the leaves. Faria *et al.* (1996) reported that the concentration of total chlorophyll showed a small increase in elevated CO₂ plant on leaf area basis, but it decreased significantly on dry weight basis as a result of the decrease in SLA in elevated plants. The changes in SLA may results from effects on leaf thickness (fresh weight/area) or changes in tissue density (dry weight/fresh weight).

According to Bunce (1997) increase in the relative growth rate (RGR) at elevated carbon dioxide were more highly correlated with the relative increase in NAR at elevated carbon dioxide than with the response of the leaf area ratio (LAR). Tognetti and Johnson (1999) reported that elevated CO₂ significantly decreased the specific leaf area (SLA) and leaf area ratio (LAR) in leaves of *Quercus ilex* L. Ziska (2002) reported that CO₂ enrichment significantly increased the biomass accumulation and the relative ratio of biomass increase to leaf area expansion in *Cirsium arvense*. The LAR and SLA under elevated CO₂ were significantly reduced compared to ambient.

Physiological Processes

Photosynthesis

Higher level of atmospheric CO₂ concentration stimulates the photosynthesis particularly C₃ plants since both CO₂ and O₂ compete for the same site on catalyzing enzyme rubisco and under elevated CO₂ condition rubisco tend to near saturated condition (Goudriaan, 1990 and Drake *et al.*, 1997). The CO₂ concentrating mechanism of C₄ plants allows CO₂ to compete more effectively with O₂ for binding sites on the enzyme, ribulose biphosphate carboxylic oxygenase (RuBP) (Allen *et al.*, 1994). Therefore, C₄ plants have a more efficient photosynthetic apparatus than C₃ plants (at temperatures above 25 °C) under recent atmospheric concentrations of CO₂ (320-355 μmol mol⁻¹) and do not increase photosynthetic rates as much as C₃ plants in response to increasing levels of CO₂ (Ziska and Bunce 1997 and Ziska, 1999).

The magnitude of the photosynthetic/or growth response to CO₂ depends on species and environment (Oberbaure *et al.*, 1985). Radin *et al.* (1987) found that in fully irrigated plants CO₂ enrichment to 650 µL L⁻¹ increased the average mid day photosynthesis in cotton by 77 per cent, but in water stressed plants, the photosynthesis was 10-15 per cent less than that of the unstressed plants. Campbell *et al.* (1988) observed an enhanced capacity of leaflet photosynthesis in soybean (*Glycin max* L. var. Bragg) when the plants were grown at 660 compared to 330 µL L⁻¹ CO₂, up to 150 per cent greater when measured during pod filling stage. Canopies of perennial rye grass (*Lolium perenne* L. cv. Vigor) grown at 600 ppm CO₂ showed a higher maximum photosynthesis rate of 77 per cent than the 350 ppm CO₂ grown one, if expressed on ground area basis (Nijs *et al.*, 1988a). The cause of increase was the development of larger canopy leaf area. Nijs *et al.* (1989a) also observed that in white clover (*Trifolium repens*) photosynthetic stimulation by high CO₂ was stronger at canopy level (103%) than for leaves (90% in full sunlight) as a consequence of foliage area development.

Andreeva *et al.* (1989) observed that mustard plant (*B. juncea* L.) under higher CO₂ had greater net assimilation and photosynthetic rates during vegetative phase than the plants grown under normal CO₂. They noted that the increase in photosynthesis occurred without additional synthesis of the fraction I protein and with the concurrent enhancement of N metabolism, plant growth and biomass accumulation. The excess of photosynthate was accumulated in chloroplast in the form of starch, which depended on plant growth temperature, for e.g. at 20/17 °C, the level of starch accumulation reached 34.4 per cent, at 27/24 °C it was 25 per cent. They concluded that high starch level in leaves is not a cause of the suppression of photosynthesis unless the excess starch damages chloroplast structure. Yelle *et al.* (1989) showed that the decline in photosynthesis of *L. esculentum* was not caused by a build up of starch but due to decline in activated Rubisco. Maevskaya *et al.* (1990) reported that during vegetative growth phase in mustard plant (*B. juncea* L.) in high CO₂ resulted in greater intensities of photosynthesis and N metabolism in the leaf. They concluded that under high CO₂,

plant homeostasis was maintained, protein and chlorophyll synthesis were not disturbed and growth and biomass accumulation increased.

The enzyme sucrose phosphate synthase (SPS) may be associated in the regulation of carbon partitioning either through activation by glucose-6-phosphate or by inhibition through Pi. Vassey *et al.* (1991) studied SPS activity in leaves of *Phaseolus vulgaris*, subjected to water stress and various CO₂ and light treatments. They concluded that effect of water stress on SPS activity was a consequence of the inhibition of photosynthesis caused by stomatal closure. This lowering of photosynthesis rate in the high CO₂ grown plant was correlated with reduced activity of SPS.

Seedlings of yellow poplar (*Liriodendron tulipifera* L.) and white oak (*Quercus alba* L.) showed an increase in carbon exchange rate (CER) by 60 and over 35 per cent, respectively compared to ambient grown seedlings (Wullschlegel *et al.*, 1992a). He *et al.* (1992) measured a 151 per cent increase in photosynthesis of Kentucky blue grass (*Poa pratensis* L.) a C₃ species under conditions of elevated atmospheric CO₂, but there was no effect on (*Andropogon gerardii*) C₄ grass.

Webber *et al.* (1994) suggested that the differences in response to CO₂ may be related to sink capacity. Idso *et al.* (1994) have reported a 118 per cent increase in CER in FACE grown cotton plants, which were exposed to a PPFD of 200 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Van Oosten *et al.* (1994) observed in tomato plants that chlorophyll a : b ratio decreased significantly over 9 days of exposure to elevated CO₂. Ziska and Bunce (1995) exposed three soybean cultivars (Maple Glen, Clrakans, CNS) to three CO₂ concentrations and three-growth temperature and observed a stimulation of single leaf photosynthesis at higher concentration of CO₂ in all cultivars.

The up-regulation or down-regulation of photosynthesis may involve anatomical as well as biochemical responses (Woodwell, 1995). For instance, photosynthetic capacity per unit leaf area may be increased as a result of increasing leaf thickness accompanied by production of an additional layer of palisade cells, as observed in soybean (*Glycine max* L.) (Hofstra and Hesketh, 1975 and Vu *et*

al., 1989). The capacities of C_3 species to respond to CO_2 dose differ, and it may be related to the species' ecological niche. For an approximate doubling of the CO_2 concentration, growth of C_3 species was stimulated on an average by 41 per cent. Crops, which tend to be C-strategy species, increased more in dry weight than wild species (58 vs. 35%). Fast-growing wild species were stimulated more than the slow-growing ones (54 and 23%, respectively). Elevated CO_2 significantly stimulated net photosynthesis in poplar hybrids (Ceulemans *et al.*, 1997 and Rodney *et al.*, 1997). Makino and Mae (1999) reported that the response of leaves to an elevated CO_2 concentration may depend on the inherent sink strength of a species.

Vaccari *et al.* (2001) investigated photosynthetic capacity of potato (*S. tuberosum* cv. Primura) leaves together with the impact of elevated CO_2 concentration on assimilation during leaf senescence in a FACE. According to them the photosynthetic capacity was not affected by elevated CO_2 as shown by the response of carboxylation capacity (V_{cmax}) and electron transport capacity (J_{max}) calculated from A/Ci curves and this lack of acclimatization response was due to the existence of a large carbohydrate sink (tuber). Nevertheless, a temporary accumulation of total non-structural carbohydrates (TNC) occurred in the leaves of plants grown under elevated CO_2 , which reflected a transient imbalance between CO_2 fixation and carbohydrate export from the leaves. Elevated CO_2 increased the rate of leaf senescence and led to a faster decrease in photosynthetic capacity of leaves. It is hypothesized that earlier leaf senescence occurred either as a result of higher leaf temperature measured in the FACE plants or by the more rapid depletion of carbohydrate storage capacity in the tubers, or both.

Liu *et al.* (2002) reported that regardless of N supply, Sitka spruce (*Picea sitchensis*) grown in elevated CO_2 had lower photosynthetic rates on a leaf area basis. Photosynthesis reduction was accompanied by a decline in Rubisco activity and leaf N concentration. Under elevated CO_2 , added N elevated photosynthesis and Rubisco activity, suggesting a dependence on N availability of the photosynthetic response to elevated CO_2 . Stomatal conductance of trees grown with added N

decreased in response to elevated CO_2 . This may account for the larger reduction in intercellular CO_2 concentration, and hence photosynthesis in the trees supplied with N than in those without N supply.

Morinaga (2002) studied the effects of long-term carbon dioxide (CO_2) enrichment on photosynthetic rate (Pn), Rubisco activity, chlorophyll content and carbon accumulation in leaves of Satsuma mandarin (*C. unshiu*) trees with and without fruit load. The Pn of whole Satsuma mandarin trees at $1000 \mu\text{L L}^{-1} \text{CO}_2$ for the first 13 days increased significantly, up to about 200 per cent, compared to that of trees at a normal atmospheric CO_2 ($360 \mu\text{L L}^{-1} \text{CO}_2$). Thereafter, Pn began to decrease gradually until it reached 170 per cent on the 35th day. The Pn of the bearing trees remained higher than those of non-bearing trees. Although there was no difference in the chlorophyll content, the bearing trees had lower specific leaf weight (SLW) and carbon content in the leaves. It seemed that the decrease in Pn during the long-term exposure to the elevated CO_2 concentration was partly due to a decrease in Rubisco activity and/or an accumulation of photo-assimilates in the leaves. The sink capacity of fruit may be important for the maintenance of Pn in long-term exposure to an elevated CO_2 concentration because the non-bearing trees had a particularly rapid decrease in Pn.

Wullschleger *et al.* (1992) observed the reduction in chlorophyll and accessory pigments under elevated CO_2 condition. The reduction in chlorophyll in yellow poplar was 27 per cent and in white oak seedlings was 55 per cent. The chlorophyll concentrations of poplar (chl a, chl b and total chl) were decreased in elevated CO_2 treatment as compared to ambient treatment (Ceulemans, 1997). Decrease in chlorophyll content due to CO_2 enrichment was also observed in *Brassica* (Rabha and Upreti, 1998; Upreti and Mahalaskshmi, 2000).

In the face of different mode of photosynthesis and plant species diversity, the primary mechanism on the effect of enhanced CO_2 on photosynthesis is yet to be precisely characterized.

Photosynthetic acclimation

Several mechanisms have been proposed to explain the down regulation of photosynthesis rates occur during acclimation (Stitt, 1991). One mechanism is that the enhanced starch accumulation in high CO₂ grown plants resulted in large grains that disrupt chloroplast membrane structure and function. A second mechanism is that plant grown under elevated CO₂ results in reduction in stomatal conductance, which restricts the amount of CO₂ entering the leaf and consequently fall in photosynthetic rate (Murray, 1995). Yet third mechanism suggests that the decrease in photosynthesis during acclimation is a consequence of enhanced rate of sucrose synthesis that accompany increased CO₂ uptake. Enhanced sucrose production results in feedback regulation of sucrose phosphate synthetase (SPS) and sequestering of Pi pools in the cytosol. Without sufficient levels of Pi returning to the chloroplast, RuBP regeneration and hence photosynthesis is suppressed and so-called feed back inhibition takes place (Stitt, 1991; Sage 1994 and Webber, 1994).

Yelle *et al.* (1989) discussed the down regulation of leaf photosynthesis on the basis of reduction in the Rubisco activity, however, the rate of photosynthesis under elevated CO₂ remained higher even the activity of Rubisco was reduced. Even at 50 per cent reduction in the Rubisco content, the increase in photosynthesis remained prevalent under elevated CO₂ at temperatures above 30 °C (Long, 1991), where as Rubisco controls the photosynthesis at ambient CO₂ concentration.

Sustained photosynthetic enhancement under elevated CO₂ requires maintenance of active sinks (Sitt, 1991; Ziska and Bunce, 1994). Photosynthetic acclimation of C₃ plant to elevated CO₂ may involve reallocation of N away from RuBP carboxylase/oxygenase (Rubisco) in to light driven reactions and/Pi regeneration enzymes (Makinow and Osmond, 1994). This kind of CO₂ induced reallocation of N is expected to cause greater effect on the photosynthetic responses to CO₂ in C₃ compared to C₄ species as the former invest considerably more N in carboxylating enzymes (Conroy, 1992). Such adjustment of N resources within chloroplast may aggravate the Rubisco limitation of photosynthesis at elevated CO₂

and is evident under nitrogen limiting condition (Morgan *et al.*, 1994). Lower photosynthetic activity on 21-day *Paspopyrum smithi* plants grown under condition of nitrogen limitation has been observed by Read *et al.* (1997). Accumulation of carbohydrates may lead to the repression of photosynthetic gene expression and excess starch seems to hinder CO₂ diffusion (Makino and Mae, 1999).

Signora *et al.* (1998) reported that over expression of sucrose phosphate synthase in *Arabidopsis thaliana* results in increased foliar sucrose/starch ratio and favors decreased foliar carbohydrate accumulation in plant after prolonged growth with CO₂ enrichment in *Arabidopsis thaliana*. Andreeva *et al.* (1989) observed in mustard plant (*B juncea* L.) under higher CO₂ that high starch level in leaves is not a cause of suppression of photosynthesis unless the excess starch damage chloroplast structure.

Long-term exposure to a doubling in CO₂ concentration lead to a variety of acclimation effects that directly or indirectly influence the photosynthetic capacity of the plant. In addition to changes in photosynthetic biochemistry and stomatal physiology, acclimation has been observed in the leaf area, leaf area index, leaf area duration, leaf thickness, branching, tillering, stem and root dry weights, fruit size, timing of developmental events, and life cycle completion (Bazzaz, 1990; Bowes, 1993 and Bowes, 1996).

Decrease in the rubisco protein content are observed in some plant as much as 60 per cent; indicating that N is being reallocated. However, rubisco protein may still decline in the presence of adequate N supplies, and the reduction is not always sufficient to account for the lower N concentration (Rowland-Bamford *et al.*, 1991; Conroy and Hocking, 1993).

High carbohydrate concentrations results in decreased transcription of Rubisco large subunit gene (Knapp *et al.*, 1993). It may be that decrease in photosynthesis in response to elevated CO₂ occurred only in circumstances where the extra carbohydrate generated by increased photosynthesis accumulates in the leaves. Repression of photosynthetic genes by increased soluble carbohydrate

concentration may explain acclimation of photosynthesis to elevated CO₂ concentration (Nie *et al.*, 1995).

A decrease in Rubisco content or its activation state is commonly found in many C₃ plants grown under CO₂ enrichment (Drake 1997). However, this cannot be a determinant for the down regulation of photosynthesis by CO₂ enrichment, because CO₂ enriched photosynthesis is not limited by Rubisco capacity but by electron transport or Pi regeneration capacity. Therefore, if the down regulation of photosynthesis occurs during long term CO₂ enrichment, either electron transport components or key enzymes of starch and sucrose synthesis should decrease (Makino and Mae, 1999).

Kubiske *et al.* (2002) exposed *Populus tremuloides*, and *Acer saccharum* to a factorial combination of ambient and elevated atmospheric CO₂ concentrations and high-nitrogen (N) and low N soil treatments in open-top chambers for 3 years. Their main objective was to compare photosynthetic acclimation to elevated CO₂ between species of contrasting shade tolerance, and to determine if soil N or shading modify the acclimation response. Electron transport capacity ($J_{\max}$) and carboxylation capacity (V_{cmax}) generally decreased with atmospheric CO₂ enrichment in all 3 years of the experiment, but there was no evidence that elevated CO₂ altered the relationship between them. On a leaf area basis, both $J_{\max}$ and V_{cmax} acclimated to elevated CO₂ more strongly in shade leaves than in sun leaves of *P. tremuloides*. However, the apparent CO₂ x shade interaction was largely driven by differences in specific leaf area (m² g⁻¹) between sun and shade leaves. In *A. saccharum*, photosynthesis acclimated more strongly to elevated CO₂ in sun leaves than in shade leaves on both leaf area and mass basis.

Although all of these mechanisms may describe the acclimation response, recent evidence has suggested that none can fully explain the long-term decreases in photosynthetic rates that characterized the acclimation phenomenon.

There are some species showing little or no down regulation of photosynthesis even when they are growing during long-term CO₂ enrichment and have excess

carbohydrates. For example potato (Sage *et al.*, 1989) and reddish (Usuda and Shimogawara, 1998) do not show any down regulation of photosynthesis where as they genetically belong to starch accumulating species. These species have the tuber or roots, which can act as large sink for carbohydrate. In radish, the biomass of the storage roots was markedly enhanced under CO₂ enrichment and consequently no over accumulation of carbohydrates was observed in leaves (Usuda and Shimogawara, 1998). Similarly in rice, the leaf sheaths can act as temporary sink for carbohydrates, and the absolute amount of carbohydrate in the leaf blades are considerably small (Nakano *et al.*, 1998). Therefore, the down regulation of photosynthesis in rice may be small compared to bean (Sage *et al.*, 1989).

Increase in light harvesting phenomenon (fluorescence study) showed greater response in B oxycamp hybrid with greater sink compared to B. oxyrrhina (Upreti *et al.*, 1998). Similarly, in rice 5th and 6th leaf serve greater accumulation of high molecular weight protein responsible for light-harvesting complex compared to reduction observed in the rubisco protein (Upreti *et al.*, 2002).

Herrick and Thomas (2001) reported that no photosynthetic down regulation in sweet gum tress (*Liquidambar styraciflua* L.) after three years of CO₂ enrichment. According to them trees rooted in the ground did not exhibit symptoms of photosynthetic down regulation as quickly as tree seedlings growing in pots. There was strong sustained enhancement of photosynthesis by CO₂ enrichment where by light saturated net photosynthesis of sun leaves was stimulated by 63 per cent and light saturated net photosynthesis of shade leaves was stimulated by 48 per cent.

Photosynthate partitioning

Effect of an elevated CO₂ concentration and a nonstructural carbohydrate status on partitioning was poorly understood from a mechanistic perspective. Evidence existed suggesting that, in elevated CO₂ levels, there is, greater photosynthesis and nonstructural carbohydrate levels, can result in a relatively greater partitioning of carbon to roots versus shoots (Roger *et al.*, 1992 and Roger

et al., 1994). Increased root growth under elevated CO_2 conditions might permit water extraction from a greater soil volume, which could be important in a future warmer and drier environment. Plants in high- CO_2 -level environments have large nonstructural carbohydrate stores and increased ratios of root to shoot (Woodwell *et al.*, 1995).

Hrubec *et al.* (1985) noted that in soybeans, grown for 2 weeks in $1000 \mu\text{L L}^{-1} \text{CO}_2$ there was a higher foliar nonstructural carbohydrate level (e.g. sucrose and starch) compared with CO_2 normal plants. The level of NSC was equivalent to $174 \text{ mg glucose g}^{-1} \text{DW}$ with elevated CO_2 as compared to $84 \text{ mg glucose g}^{-1} \text{DW}$ in control plant leaves. They also found a strong positive correlation between respiration rate and carbohydrate content in CO_2 adapted plant. Relatively higher starch accumulation occurred in the chloroplast of mustard leaves grown under elevated level of CO_2 (Andreeva *et al.*, 1989). Roland-Bramford (1990) grew rice plant under different CO_2 concentration and found that the ratio of starch to sucrose concentrations was positively correlated with CO_2 concentration

Bhattacharya (1992) noted that elevated CO_2 in the atmosphere results in increased concentration of carbohydrate and dilution of other metabolites like chlorophyll, proteins, amino acids and nutrients in plant tissue. Non-structural carbohydrate concentration of foliage in *Pinus palustris* was increased by elevated atmospheric CO_2 and decreased by increased N availability (Mitchell, 1995).

Faria *et al.* (1996) reported that the accumulation of soluble sugars was 44 per cent and that of starch was 58 per cent in leaves of *Quercus puber* grown under elevated CO_2 condition. Elevated CO_2 significantly increased sucrose and starch concentration in leaf of *Quercus robur* seedlings (Picon *et al.*, 1997).

Wang and Nobel (1996) observed that glucose and starch in chlorenchyma of basal cladode of *O. ficus indica* increased by 175 and 57 per cent respectively at elevated CO_2 condition when compared to current CO_2 concentration of $370 \mu\text{mol mol}^{-1}$, but sucrose content was virtually unaffected. Doubling CO_2 concentration increased the malate production in basal cladodes by 75 per cent, Pi by 32 per

cent, soluble starch synthase activity by 30 per cent and sucrose-Pi synthase activity 146 per cent, but did not affect the activity of hexokinase. Doubled CO₂ accelerated phloem transport of sucrose out of the basal cladodes, resulting in a 73 per cent higher dry weight for the daughter cladodes. Doubling CO₂ increased the glucose content in 14 d daughter cladode by 167 per cent. No downward acclimation of photosynthesis under elevated CO₂ was observed.

The carbohydrate concentration in source leaves was increase in elevated CO₂, partially at low N supply. Elevated leaf carbohydrate concentration was related to increase structural carbon (C) to nitrogen (N) ratio and thus reflected an increase C availability together with N dependent sink limitation (Fischer *et al.*, 1997). Habeisen *et al.* (1997) reported that the yield response of *L. perenne* was strongly dependent on N fertilizer in FACE experiment. Rabha and Uprety (1998) reported that elevated CO₂ enhanced the allocation of biomass to the roots, which serve as strong sink for assimilated carbon.

Palta and Ludwig (2000) exposed the indeterminate narrow-leaf lupin (*Lupinus angustifolius* cv. Merrit) to enriched atmospheric CO₂ during pod filling. They observed that CO₂ enrichment increased in the final number of pods and the number of pods to zero that filled large seeds (150 mg) by 20-22 pods/plant. Enriched CO₂ reduced the number of pods that had small seeds (30-80 mg) and reduced the number of pods with unfilled seeds from 16 to 1 pod/plant. This resulted in increase seed yield per plant by 44-66 per cent. Harvest index was unchanged under enriched CO₂, while increasing pod filling also increased pod set and dry matter accumulation on the developing branches proportionally. This indicated that an increased availability of carbon resources during-pod filling changed the allocation of assimilates by filling small seeds and producing new branches. The 47-56 per cent increase in dry matter per plant was reflected in the increase in seed yield, which occurred largely through an increased number of pods and seeds per plant. These data support the idea that seed-filling and hence seed yield in well nodulated, was limited by carbon resources at the stage when

the lupin plant was most source-limited, i.e. during pod set and pod filling.

Chen (2002) reported that the elevated CO_2 concentration increased carbohydrate production of apple trees. Both content and accumulation of non-structural carbohydrates, particularly starch and sucrose, increased in all organs of the trees under the doubled ambient CO_2 condition. Relative distribution of non-structural carbohydrates within the trees rose in leaves, shoots, and roots, but declined in stems due to the long-term atmospheric CO_2 enrichment. Carbohydrate proportions and starch: sucrose ratio in different organs of the trees varied in line with the duration of the experiment. When compared with 350 ppm CO_2 treated trees, those grown at 700 ppm, CO_2 increased non-structural carbohydrate-production efficiency of leaves by 48 and 32 per cent in the first and the second 60 days of the CO_2 treatments, respectively. Long-term atmospheric CO_2 enrichment constantly improved non-structural carbohydrate-production efficiency of the apple trees, especially starch-production efficiency. A further increase of the global atmospheric CO_2 concentration in the future will promote carbohydrate production of apple trees and consequently enhance the fruit productivity.

As shown in this part of review, the fate of photosynthate was determined through partitioning, accumulation and metabolism associated in the growth of different plant organs. This raises one simple question: how does the additional C obtain from greater CO_2 fixation influence the growth at organ and whole plant level?

Respiration

Much research of plant response to elevated CO_2 have focused on photosynthesis and growth in crops and trees but little attention has been given to understanding respiratory responses even though respiration may consume more than 50 per cent of the C fixed at the whole plant level (Farar, 1985 and Amthor, 1989) and despite evidence suggesting that CO_2 may directly influence respiration (Amthor, 1991 and Wullschlegel *et al.*, 1994). It has been demonstrated that

respiration was markedly sensitive to atmospheric CO₂ (Amthor, 1991). Thus, photosynthesis rate also depended on other process, principally photorespiration and change in respiration even to cause large variations in dry matter production (Amthor, 1989 and Amthor, 1994).

Gifford *et al.* (1985) observed a 45 percent reduction of the specific respiration rate in roots of CO₂-enriched wheat (*Triticum aestivum* L.). Bunce (1990) reported that whole plant CO₂ efflux rates per unit mass were lower in soybean and tomato plants grown at higher CO₂ pressure (70 Pa). Bunce and Caulfield (1991) observed a 30-40 percent reduction in CO₂ efflux rates in *L. perenne* and *Medicago sativa*, grown under controlled environmental elevated CO₂.

DuCloux *et al.* (1989) studied the effect of doubling of atmospheric CO₂ concentration on photosynthesis of wheat; they observed that O₂ uptake was reduced by 40 percent by high CO₂. Wullschleger and Norby (1992) have noted that growth respiration decreased by 12-31 per cent and maintenance respiration decreased by 45 to 54 percent for sapling leaves grown at elevated compared to with an ambient CO₂.

Baker *et al.* (1992) subjected rice plants (*Oryza sativa* cv. IR-30) in naturally sunlit plant growth chamber for entire season in sun-ambient (160 and 250 µmol mol⁻¹), ambient (330 µmol mol⁻¹) or super-ambient (500, 660 and 900 µmol mol⁻¹) CO₂ treatments. They reported that specific respiration rate decreased with time during the growing season and was higher in sub-ambient than ambient and super-ambient CO₂ treatments. This greater specific respiration rate in sub-ambient condition was attributed to a higher specific maintenance respiration rate and was associated with higher plant tissue N content. In rice, the effects of elevated CO₂ on dark respiration were observed throughout the growth cycle (Baker *et al.*, 1992 and Upreti *et al.*, 2002)

Working on Australian bottle tree species (*Brachychiton populneum*) and two Eucalyptus (*E. microtheca* and *E. polyanthemus*), Idso and Kimball (1993) showed that dark respiration dropped by approximately 50 per cent for a 360-720

$\mu\text{L L}^{-1}$ doubling of the air's CO_2 concentration, while net photosynthesis rose by a factor of 2.0 in Australian tree species. Azicon-Bieto *et al.* (1994) measured dark O_2 uptake in leaves and stem of C_3 and C_4 plants grown at elevated and ambient CO_2 condition. Elevated CO_2 decreased the O_2 uptake in stem of *S. olneyi* and leaves of *Lindera benzoin* when expressed on either a dry weight or area basis.

In an experiment with soybean plants on CO_2 enrichment Bunce (1995a) noted decline in respiration rate. He also observed that response of respiration rate to CO_2 was not affected by the form of N. He showed that high CO_2 ($700 \text{ cm}^3 \text{ m}^{-3}$) applied only during night conserved C and increased dry matter of soybean during initial growth compared with the constant $350 \text{ cm}^3 \text{ m}^{-3}$ CO_2 treatment. Long-term net assimilation was increased by high CO_2 in the dark without any increase in day by the high CO_2 in the dark to values equal to those of plants continually exposed to higher CO_2 concentration (Bunce 1995b).

Bunce and Ziska (1996) observed that respiration of soybean grown in higher CO_2 concentration, acclimated to increased growth temperature by reducing maintenance respiration, and that elevated CO_2 did not persistently increase RGR but had the direct effect of reducing respiration. Growth of plants under conditions of elevated CO_2 often results in a decrease in the dark respiration rate (Amthor, 1991, 1997). Dark respiration is inhibited on an average by 15-18 per cent by a doubling of present atmospheric CO_2 concentration (Amthor, 1997; Drake *et al.*, 1997; Curtis and Wang, 1998).

The rapid inhibitory effect of elevated CO_2 on efflux rates could be related to inhibition of the activity of some respiratory enzymes e.g. Succinate dehydrogenase (Shaish *et al.*, 1989) or cyt. c oxidase (Gonzalez-Meler *et al.*, 1996). Cytochrome C oxidase (COX) was considered a likely candidate for the primary effect of CO_2 in the respiration. Gonzalez-Meler *et al.* (1996) found in vitro COX activity was reduced to 19 per cent by an increase in CO_2 ($360 \mu\text{mol mol}^{-1}$) concentration. However, more recently Gonzalez-Meler and Siedow (1999) and Bunce (2001) suggested that the primary effect of CO_2 is not on COX. This is because typical

in vivo flux control coefficient of COX (in absence of ADP) are near zero (Gonzalez-Meler and Siedow, 1999) and because of elevated CO₂ inhibits respiration to a greater extent in substrate availability (Bunce 2001). Atkin *et al.* (2000) in *E. pauciflora* and Bruhn *et al.* (2002) in *Plantago* species, found that the relative difference between rates of respiration at two atmospheric CO₂ values increased with decreasing temperature. Elevated CO₂ may also influence leaf respiration indirectly by altering growth rate, nonstructural carbohydrate concentration and other changes in tissue composition (Hamilton *et al.*, 2001).

Carbon nitrogen (C/N) ratio

The elevated levels of CO₂ increased the carbon sequestering in the ecosystem and simulated plant growth leading to dry matter accumulation, which may change, based on nutrient availability. It is believed that in environment of rising atmospheric CO₂, the availability of nutrients may ultimately limit the capacity of plants to assimilate carbon (Riviere-Rolland *et al.*, 1996). The expected higher plant growth under higher CO₂ concentrations would improve increased demand upon nutrients, so that N, S or P could be among the first limiting factors (Lemon, 1983). The CO₂ enrichment can improved the NUE of some C₃ species (Goudriaan and de Ruieter, 1983).

The capacity to generate sinks is dependent not only up to species but was also a function of the soil nutrient supply (Radin and Mauney, 1982). Norby *et al.* (1986) observed that there was enhancement of growth by 85 per cent of soybean plants, which were grown under elevated CO₂ concentration, but these plants were severely N deficient. Sink strength mediated through N supply is crucial in determining the growth responses of plants to increase in atmospheric CO₂ concentration (Allen *et al.*, 1988).

With higher CO₂ there was increase in NUE and decrease in concentration of N, S and B per unit tissue (Wong, 1990). There was significant overall decrease in leaf N content at higher levels of CO₂ (Penuelas and Matamala, 1990). The nitrogen concentration of foliage and roots were found to be consistently lower

in plants growth at elevated CO_2 , irrespective of availability of N in soil (Hocking and Meyer, 1990). The virtual disappearance of any effect of CO_2 on the shoot N concentration when N is freely available indicated the involvement of an uptake limitation, possibly related to the flux of NO_3^- to the root surface (Vassey *et al.*, 1991). They estimated that the root-derived carbon accounts for between 10 and 40 per cent of the total assimilated carbon.

Reduction in tissue N concentration at high CO_2 grown plants might indicate physiological changes in the efficiency with which plants use N to gain biomass (Hilbert *et al.*, 1991). Hocking and Meyer (1991) working on *Triticale* reported that CO_2 enriched plants had lower concentration of N in their leaves and increased NUE than controls. Decline in ear N or grain N was detected by Hocking and Meyer (1991). The C/N balance of plants is therefore, responsive to the CO_2 level of the atmosphere during growth and development (Stitt, 1991).

Conroy (1992) reported that high CO_2 alters nutrient requirement and N concentration of foliage, roots and grains are consistently lower in plants grown at elevated CO_2 , irrespective of availability of N in soil. According to him, changes in nutrient uptake from the soil, remobilisation in the plant or efficiency of utilization at elevated CO_2 will influence fertilizer management in agricultural and forest ecosystems and nutrient cycling in natural ecosystem.

The C/N ratio of plants grown in elevated atmospheric CO_2 was increased (Curtis *et al.*, 1989 and Baker *et al.*, 1992). Conroy and Hocking (1993) suggested that stomatal closure resulting in decreased transpiration rates in CO_2 enriched atmosphere is in part responsible for the decrease in N content of plant grown in these conditions. Nitrogen mineralization could increase response of labile C inputs for root production and this carbon becomes key regulator of soil dynamics (Clarton, 1995). Bunce & Ziska (1996) observed that at doubled CO_2 concentration, the N content of soybean was lowered by 24 per cent.

Ziska *et al.* (1996) noted that the photosynthesis became dependent on N in later growth stages in rice, which were grown at ambient and elevated CO_2

conditions at 3 levels of supplemented N. On a total dry weight basis, the leaf N concentration in cotton was reduced by 33 per cent at CO₂ concentration of 550 $\mu\text{mol mol}^{-1}$ compared with those grown at ambient CO₂. The CO₂ effect diminished as N supply was increased until at the highest N supply (133 mg N kg⁻¹ soil week⁻¹), there was no significant depression in leaf N concentration. Dilution of leaf N by non-structural carbohydrate contributed to but did not fully account for the observed reduction in leaf N concentration (Rogers *et al.* 1996). The total N in the cotton leaves growing with elevated CO₂ were reduced (Wong, 1993). Read *et al.* (1997) observed a 26 per cent reduction in leaf N concentration due to higher concentration of CO₂. Elevated CO₂ caused a concomitant decrease in the N content of leaves in pot grown tobacco (Merry, 1997). Higher atmospheric CO₂ levels may lead to quantitative and qualitative shifts in carbon and nitrogen fluxes within plants and soil (Van Ginkel *et al.*, 1997).

Tognetti and Johnson (1999) exposed *Quercus ilex* at two CO₂ levels (370 or 520 $\mu\text{mol mol}^{-1}$) in combination with two soil N treatments (20 and 90 $\mu\text{mol mol}^{-1}$ total N) in OTC they found that Leaf N concentration decreased significantly in elevated CO₂ irrespective of N treatment. As a result of patterns of N and carbon concentrations, C/N ratio generally increased with elevated CO₂ treatment and decreased with high nutrient supply. Starch concentrations in leaves, stem and roots increased with elevated CO₂ and decreased with nutrient addition. Williams *et al.* (2000) reported that sugar maple and red maple grown at elevated CO₂ concentration were significantly reduced in leaf nitrogen concentration and increased C : N ratio. Uprety and Mahalaxmi (2000) reported that the elevated CO₂ increased carbon sequestration in leaf, stem and roots of *Brassica* plants through increased photosynthesis and decreased respiration. According to them application of additional nitrogen nutrition sustain the positive effect of CO₂ enrichment by utilizing additional carbohydrates for the development of new sink. Excess carbohydrates make plants flexible and responsive to additional nitrogen application for sustaining this CO₂ enrichment.

The plant and soil related controls of carbon and nitrogen interaction in the rhizosphere are still poorly understood and need to be classified (Griffin *et al.*, 1993). Therefore, predicting the response of plants with higher level of atmospheric CO₂ require an understanding of the interaction between CO₂ and limiting environmental factors such as nutrient availability especially nitrogen.

Stomatal sensitivity

Out of very few direct effect of CO₂ on plant morphogenesis, opening and closing of stomata is important as it affects both the process of photosynthesis and the water use. It is widely observed that stomatal aperture for many species of both C₃ and C₄ metabolism is sensitive to CO₂ concentration (Morison and Gifford, 1983).

Morison and Gifford (1984) also observed that a doubling of CO₂ decreased stomatal conductance by approximately 36 per cent. However, lower stomatal conductance has a minor effect on photosynthesis because the gas diffusion is more rapid than the photochemical uptake of CO₂.

Warrick *et al.* (1986) reported that a 40 per cent reduction in stomatal conductance with a doubling of CO₂ concentration. This response applies equally to C₃ and C₄ species. Guard cells respond rapidly to relatively small changes in nearby CO₂ concentration and are believed by some to be unique among plant cell types in their ability to sense these change (Mott, 1990).

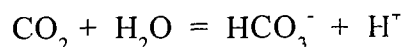
Knapp *et al.* (1994) demonstrated a 50 per cent reduction in stomatal conductance at elevated CO₂ in *A. gerardi* leaves. Upreti *et al.* (1995) observed that *Brassica* species responded to elevated CO₂ significantly under moisture stress conditions mitigating the adverse effect on photosynthesis and growth. The water status of plant significantly improved under elevated CO₂ concentration possibly by increasing stomatal resistance and/or increased root growth.

Heath and Kerstiens (1997) observed a lower stomatal conductance in CO₂ enriched air in Oak where as the high nutrient treatment consistently reduced

stomatal closer. Han *et al.* (1999) studied the water use efficiency (WUE) of *Pinus sylvestris* var. *Sylvestris* in response to increased CO₂ concentration (500±100 µL L⁻¹). Increased CO₂ concentration reduced average stomatal opening, stomatal conductance and stomatal density to 78, 80 and 87 per cent, respectively, compared with their values under normal ambient CO₂.

Light and CO₂ interact in regulating stomatal movement, with higher CO₂ concentration modifying and overriding the light effect (Murray, 1995). The extent of opening is greater as light intensity increases or as CO₂ concentration decreases (Willmer, 1983). The CO₂ sensing mechanism of stomata is effective that a stream of CO₂ free-air will promote stomatal opening even in darkness. Conversely, a high external concentration of CO₂ restricts opening, even at high light intensity. Water deficit overrides both light and CO₂ concentration, triggering stomatal closure (Murray, 1995).

The mechanism of stomatal opening, involving changes in turgor brought about by the influx of K⁺ ions in exchange for H⁺ ions, is relatively well characterized (Zeiger *et al.*, 1987). The mechanism, how CO₂ interacts with this process is unclear. The pH effects are thought to be insignificant and the photosynthetic C reduction pathway appears to be absent in guard cell (Outlaw, 1989). However, being one immediate product of CO₂ metabolism, H⁺ might be 'sensed' by virtue of carbonic anhydrase reaction (Murray, 1995):



Guard cells are almost always the only epidermal cells to possess chloroplast and these chloroplast do not lack Rubisco activity as previously thought of. CO₂ is involved in the mechanism of stomatal opening, being required for PEP carboxylation to oxaloacetate, which is converted to malate by the irradiance-inducible enzyme NAD-malate dehydrogenase (Zeiger *et al.*, 1987; Birkenhead and Willmer, 1986). CO₂ in some way may inhibit the plasmalemma H⁺ extrusion pumps, or some buffering effects involving CO₂/carbonate/bicarbonate interchange may be involved (Gotow *et al.*, 1985). Since there is close correlation between

photosynthetic and stomatal response to CO₂, the photosynthetic C reduction pathway in the subsidiary cells of the stomatal complex may have an intricate role (Jarvis and Mansfield, 1981), in which case, stomatal aperture would be expected to acclimate to elevated CO₂ as a results of photosynthetic acclimation. Upreti *et al.* (2002) observed that elevated CO₂ brought about a reduction in stomatal conductance and increase in stomatal index, size of stomatal guard cells, stroma and epidermal cell in rice cultivars and demonstrated that such acclimation helped in the regulation of photosynthesis to the higher elevated CO₂. According to them, these changes in stomatal characters made rice cultivars adjustable to higher CO₂ environment.

Wullschleger *et al.* (2002) in study on *Liquidambar styraciflua* (Sweetgum) observed that midday measurements of stomatal conductance for leaves exposed to free-air CO₂ enrichment were up to 44 per cent lower at elevated than at ambient CO₂ concentrations, whereas canopy conductance, averaged over the growing season, was only 14 per cent lower in stands exposed to CO₂ enrichment. The magnitude of this response was dependent on vapour pressure deficit and soil water potential. Annual estimates of evapotranspiration showed relatively small reductions due to atmospheric CO₂ enrichment.

Woodward *et al.* (2002) studied the stomatal density responses by 48 accessions of *Arabidopsis thaliana* (accessions Col-O and Ws) to CO₂ enrichment. They found that accessions differing in the degree of stomatal response to both CO₂ and drought differed in flower production. They observed that the flowering was benefited from a small reduction in stomatal density with CO₂ enrichment under well-watered conditions, however a large reduction in stomatal density under drought. Stomatal density increased with altitude in *Vaccinium myrtillus* but was also strongly influenced by exposure as observed in a field experiment. Exposed plants had higher stomatal densities than plants at the same altitude. This difference might be explained by systemic signalling within the plant as mature leaves detect both irradiance and CO₂, subsequently controlling the response of stomatal

development in developing leaves. Plants with the highest stomatal densities also had the highest stomatal conductance and photosynthetic rates.

Sebastiani *et al.* (2002) exposed one-year-old olive (*Olea europaea*) cuttings of two cultivars (Frantoio and Moraiolo) for five months to ambient ($360 \pm 16 \mu\text{mol mol}^{-1}$) and elevated ($560 \pm 52 \mu\text{mol mol}^{-1}$) CO_2 concentration in a free-air CO_2 enrichment facility. According to them exposure to elevated CO_2 enhanced rates of net photosynthesis and decreased stomatal conductance, leading to higher water use efficiency. Chlorophyll concentrations (chlorophyll content per unit leaf area) decreased only in Frantoio leaves of plants grown in elevated CO_2 . Stomatal density and leaf nutrients did not differ between treatments and cultivars.

Water use efficiency (WUE)

The increase in water use efficiency (WUE) is one of the most significant plant responses, identified to rising CO_2 . Morison (1985) reported that increases in short-term measure of WUE in the range of 60-160 per cent for both C_3 and C_4 species with a doubling of CO_2 concentration. Nijs *et al.* (1988a) showed that transpiration declined under high CO_2 on a leaf area basis, but total plant canopy transpiration was at least as large as in ambient CO_2 due to the large leaf area in white clover. Doubling of CO_2 improved WUE by 35 per cent over the control. In *L. perenne*, Nijs *et al.* (1989b) found that, while total water use was the same under high and low CO_2 levels, WUE was increased 87 per cent on leaf basis and only 25 per cent on canopy level.

The increase WUE was as a result of narrowing of stomatal aperture that would result from an increase in the concentration of ambient CO_2 (Rogers *et al.*, 1983). King and Greek (1986) noted that the WUE increased by 34 and 55 per cent at 600 and $850 \mu\text{L L}^{-1}$ CO_2 over ambient grown maize. Elevated CO_2 improved the WUE *L. perenne* by 35 per cent over control (Nijs *et al.*, 1988). Zika *et al.* 1991 investigated nine different tropical grass species for doubled CO_2 and obtained strongly significant enhancement of photosynthesis and water use efficiency. Han *et al.* (1999) reported that increased CO_2 reduced transpiration to 76 per cent and

results in enhancement of the water use efficiency (WUE) of *Pinus sylvestriformis* plants.

Wullschleger and Norby (2001) reported that WUE of 12 year-old sweet gum tress exposed to FACE for a 7 month growing season was 1.90 g of woody biomass produced per kg water used compared to 1.48 g kg⁻¹ for trees in the ambient CO₂ treatment. This 28 per cent increased in stand level WUE was the combined result of a 15 per cent increase in dry matter increment.

Conley *et al.* (2001) observed that elevated CO₂ reduced cumulative ET by 10 per cent when Sorghum (*Sorghum bicolor*) plants were given ample water, and by 4 per cent under severe drought stress. Water use efficiency (WUE) based on grain yield increased, due to CO₂ enrichment, by 9 and 19 per cent in wet and dry plots, respectively; based on total biomass, WUE increased by 16 and 17 per cent in wet and dry plots, respectively. They suggested that in the future high-CO₂ environment; water requirements for irrigated sorghum will be lower than at present, while dry-land productivity will increase, provided global warming is minimum.

Bethenod (2001) studied the effect of elevated CO₂ concentration on plant transpiration and water use efficiency in maize (*Zea mays*). Maize plants were grown in two separate compartments, an ambient CO₂ and in the second CO₂ was enriched to about double the normal level. They observed that the daily transpiration of plants increased with leaf growth. Doubling CO₂ induced a decrease in daily plant transpiration. Over the entire crop life, the daily mean transpiration decrease was 17 per cent. The decrease in cumulated transpiration was 17 per cent too. Doubling CO₂ induced a 23 per cent increase in water use efficiency (dry weight/cumulated transpiration).

Chen *et al.* (2002) reported that the atmospheric CO₂ enrichment accelerated new leaf appearance, total leaf area increment, and total shoot length of the trees due to the increase of carbohydrate production of the two-year-old apple plants. As a result of dry mass accumulation in the plant tissues, water content in all

organs of the trees tended to decline during the long-term atmospheric CO₂ enrichment. Cumulative water consumption, however, was higher in trees exposed to 700 than 350 ppm CO₂ concentration. The mean of daily water consumption per tree was recorded 174.4, 297.2, 308.3, 231.3 and 135.7 ml for trees grown under 350 ppm CO₂ condition and 206.7, 334.5, 346.2, 277.4 and 173.4 ml for those at 700 ppm CO₂, respectively. The increase of total water consumption due to higher CO₂ was 13-16 per cent and water-use efficiency by 98-130 per cent, whereas leaf transpiration rate and water-uptake efficiency of the plant roots decreased by 27-33 per cent and 39-41 per cent. Linear regression correlation analyses revealed significantly positive relationships among cumulative sunshine duration, natural evaporation, air temperature, total leaf number as well as total shoot length per tree, and total water consumption of the trees at both 350 and 700 ppm CO₂ concentrations. According to them, the physiological mechanisms to enhance the canopy development and foliage transpiration and to raise water-consumption efficiency of the plants were associated with the altered water status and improved growth turgor of the trial apple trees treated by the long-term doubled atmospheric CO₂ concentration.

Water relations

Long-term exposure of plants to elevated CO₂ leads to a number of growth and physiological changes, which possibly ameliorated the negative impacts of drought (Upreti, 1995 and Wullschleger *et al.*, 2001). According to them, the growth of plant at elevated CO₂ can lead to conditions whereby plants maintain higher leaf water potentials. The mechanisms that contribute to this effect may probably involve reduction in stomatal conductance, increases in whole plant hydraulic conductance and osmotic adjustment.

Increase in water-use efficiency and reductions in water use can contribute to enhanced soil water content under elevated CO₂. Herbaceous crops and grassland are most responsive in this regard. Even small effects of elevated CO₂ on plant and soil water relations can have important implications for ecosystem. Upreti

et al. (1995) demonstrated amelioration of stress effect due to elevated CO₂ and explained it to the maintenance of water status through increasing water potential and more water intake by greater root growth induced at higher CO₂ concentration.

According to Centritto *et al.* (1999b) elevated CO₂ might reduce transpiration rate by causing a decline in stomatal conductance leading to increase in plant water potential and a delay in the onset of drought. Increases in fine root production under elevated CO₂ represent another mechanism where by forests might adjust to future climate. If greater rooting density/or depth is attained by forest in the future they may be able to tap new water sources and maintain normal function under altered atmospheric conditions (Hanson and Weltzin, 2000).

According to Pritchard and Rogers *et al.* (2000) atmospheric CO₂ enrichment can change the distribution of roots in the soil, which might also have implications for water uptake. Several experiments suggest that crop plants in higher CO₂ atmosphere will have larger root systems that are more highly branched, especially at shallow depth, and this should increase the capacity for resource acquisition, but at lower efficiency CO₂ enrichment of a grassland community led to an upward shift in root length density with greater proportion of roots in the top 6 cm soil layer This shift was perhaps related to increased soil moisture and its effect on nutrient availability (Arnone *et al.*, 2000).

Osmotic adjustment

According to Conroy *et al.* (1988) osmotic adjustment occurred during drought in expanded leaves of sunflowers cv. 'Hysun 30' which had been continuously exposed to 660 $\mu\text{L L}^{-1}$ CO₂ or had been previously acclimated to drought. The effect was greatest when the treatments were combined and was negligible in non-acclimated plants grown at 340 $\mu\text{L L}^{-1}$ CO₂.

Ferries and Tailor (1994) reported that three of the four chalk grassland herbs that they studied, including salad burnet (*Sanguisorrba minor Scop*), Kidney vetch (*Anthyllis vulneraria L.*) hoary plantain (*Plantago media L.*), had lower

foliar osmotic potential (by 0.3 Mpa) when grown at elevated compared to ambient CO_2 and there by maintain the plant water status.

Chaves *et al.* (1995) reported that a marginal improvement in the response of trees to drought appeared to occur under conditions of long-term CO_2 enrichment in *Quercus ilex*. This was expressed, for example, by the absence of midday stomatal closure in trees growing under increased CO_2 . Some protection against high irradiance and high temperature also seemed to occur at the photochemical level, presumably as a result of more carbon available to the consumption of excess light energy. This would allow a better performance of the plants grown under increased CO_2 during the warmer hours of the day and, therefore, play an important adaptation role under drought conditions. A marginal increase in the concentration of soluble sugars and starch was also observed in the leaves of trees growing in increased CO_2 , mainly during the midday hours. They suggested that this would be advantageous both in terms of carbohydrate reserves for growth (e.g. more roots) and osmotic adjustment. Uprety and Rabha (1999) demonstrated that non-structural carbohydrate accumulated due to elevated CO_2 in *Brassica* helped in the osmotic adjustment under moisture stress condition.

According to Ellsworth (1999) elevated CO_2 may directly alter leaf dehydration tolerance in cases where CO_2 induced excess carbohydrates serves as osmotica. Elevated CO_2 could increase drought tolerance of plants if increased rates of net carbon assimilation leads to an increased availability of substrate for osmotic adjustment, thereby lowering osmotic potential at full turgor (π_o). Elevated CO_2 could, therefore, have significant effect on drought tolerance by inducing carbohydrate accumulation and lowering π_o when soil moisture availability is high as well as by enhancing osmotic adjustment during drought (Wullschleger *et al.*, 2002).

Urban *et al.* (2002) reported that the pressure-volume and transpiration curves were established in summer and autumn on leaves of 'First Red' and 'Twingo' rose plants grown at control and at elevated CO_2 ($[\text{CO}_2]$ a=35.5 and 70 Pa, respectively). CO_2 enrichment resulted in an increase in the non-osmotic water

fraction in both summer and autumn. Osmoregulation, as evidenced by a decrease in osmotic potential at full turgor and at turgor loss, and a concomitant decrease in the relative water content at the point of turgor loss, were observed as a consequence of elevated CO_2 in 'First Red' leaves only in autumn, while the average elastic modulus was increased only in 'Twingo' leaves. These adaptive mechanisms improve the leaf capacity to take up water by lowering their water potential. The water potential at complete stomatal closure was higher under elevated CO_2 conditions in 'First Red' in both summer and autumn, and in 'Twingo' in autumn, indicating a higher leaf capacity against water loss through transpiration. The cuticular transpiration rate decreased at high CO_2 in the two cultivars.

Anatomy

Roger *et al.* (1983) reported increase in thickness of all layers of leaf of *Pinus taeda* and *Liquidambar styraciflua* grown in high CO_2 . The difference in leaf thickness was reflected in difference in the ratio of leaf weight to leaf area (Jarvis, 1985). Leadley (1987) reported that the soybean plants grown in the high CO_2 treatment had thicker leaves but less palisade cell surface area per unit leaf area. Surface area of the mesophyll per unit leaf area was unaffected by CO_2 .

Overdieck and Ungemach (1989) recorded that elevated CO_2 induced increase in density of palisade and spongy mesophyll at the expense of intercellular spaces in *T. repens* and *Lolium perenne*. Stomatal frequency in the lower epidermis was not affected by CO_2 enrichment.

Radoglou and Jarvis (1990) studied the anatomy of leaves of poplar clone Columbia River, Beaupre, Robusta and Raspalje at ambient ($350 \mu\text{L L}^{-1} \text{CO}_2$) and elevated ($700 \mu\text{L L}^{-1} \text{CO}_2$) CO_2 concentration. All four clones exhibited significant increase in total leaf thickness at the higher CO_2 concentration. This increase was 11 per cent in Columbia River, 15 per cent in Beaupre, 8 per cent in Robusta and 16 per cent in Raspalje. There was also significant difference between the CO_2 treatments in the palisade and spongy parenchyma. Both upper and lower epidermis tended to be thicker in leaves that developed in high CO_2 .

Lee *et al.* (1993) reported that when Birch has been grown from seed in ambient and elevated CO₂ for two years, leaf anatomy changed, with the addition of an extra palisade layer. Liu *et al.* (2002) reported that elevated CO₂ resulted in larger fresh mass, dry mass, and leaf area and leaf thickness in two-year old needles in young trees of Sitka spruce (*Picea sitchensis*).

The carbon induced growth effect occurred because of changes in the biophysical properties of the cell wall, particularly enhanced wall extensibility in chalk grassland herbs (Ferris and Taylor, 1994). A wall loosening enzyme, xyloglucan endotransglycosylase (XET) that alters wall structure, has been correlated with increased leaf growth in elevated CO₂ (Ranasinghe and Taylor, 1996 and Ferris *et al.*, 2001). Upreti *et al.* (2002) reported that elevated CO₂ brought about significant increase in size of stomatal guard cells, stroma and epidermal cell, and such acclimation involve in the regulation of photosynthesis.

Changes in chloroplast density or volume which occur in plants grown in CO₂ enriched environment have been generally attributed to increased starch accumulation in the chloroplasts of tomato and soybean (Madsen 1971). Thomas and Harvey (1983) examined the effect of continuous, atmospheric CO₂ enrichment on leaf anatomy and morphology in corn, soybean, loblolly pine and sweet gum. Plants were exposed to constantly CO₂ level of 340, 520, 718 and 910 ppm throughout growing season. Significant increase was measured in leaf thickness for the three-C₃ species under enriched CO₂ conditions, but not for corn, a C₄ plant. Chloroplasts in leaf tissues of plants from CO₂ enriched treatments appeared denser and were more deeply stained by crystal violet. Such increases in chloroplast density are similar to that reported in tomatoes grown under increased CO₂ level. Elevated CO₂ stimulated cell production in roots cells (Zhao *et al.*, 2000).

Bray and Reid (2002) studied the effect of CO₂ and NaCl on the second trifoliate leaf of *P. vulgaris* cv. 'Taylor'. Elevated CO₂ could partially overcome the adverse salinity effects such as leaf area, volume, specific leaf area, and relative leaf expansion rate. Leaf fresh and dry weights, leaf volume, palisade and spongy

mesophyll tissue volume, and the numbers of palisade and epidermal cells per leaf equalled controls. Under high CO₂, the volume density of the palisade mesophyll increased. Leaf thickness, palisade cell length and volume, volume density of spongy mesophyll, and succulence were greater than controls in salt and high CO₂ leaves. High CO₂ had more effect on salt-stressed than unstressed plants in leaf weight, thickness, and cell volume.

Leaf ultrastructure

Vu *et al.* (1989) showed increases of 37, 205, 108, 33, 22 and 31 per cent in specific leaf wt, starch, sucrose, reducing sugars, chlorophyll and soluble protein respectively due to elevated CO₂ (800 µL L⁻¹ CO₂) in Soyabeans cv. 'Bragg grown'. CO₂ enrichment caused an increase in leaf thickness, due to an increased number of palisade cells, but no major alteration in chloroplast ultrastructure. The extra starch formed under CO₂ enrichment was distributed as slightly larger starch grains among more and larger chloroplasts and more mesophyll cells, rather than as more starch grains per chloroplast. The increase in starch storage sites, together with the maintenance of ribulose-bisphosphate carboxylase activity, enable soybeans to continue to exhibit high photosynthetic rates throughout the growth period under high CO₂ levels.

Elevated CO₂ brought about increase in cell and chloroplast expansion of wheat (*Triticum aestivum*) leaves 10 and 25 per cent, respectively and observed that older leaves chloroplast contained larger starch deposits (Robertson *et al.*, 1995a). Robertson *et al.*, 1995b) reported that organelle changes stimulated by elevated CO₂ are not restricted to chloroplast however a dramatic stimulation in mitochondrial biogenesis during the very early stages of leaf development was observed in young wheat plants. An almost 3 fold increase in the number of mitochondria was observed in the very young leaf cells at the base of the first leaf of a 7 day old wheat plant.

Utriainen (1998) studied the effect of increased (2x ambient) O₃ and CO₂ levels, singly and in combination on (*Pinus sylvestris*), in open-top chambers. At

the ultrastructural level, O_3 caused increased electron density of the chloroplast, stroma and an increased number of cytoplasmic ribosomes at both CO_2 levels. CO_2 enrichment also resulted in an increase in the size of starch grains in the chloroplasts. In general, simultaneous exposure to increased O_3 reduced the impact of increased CO_2 . Utriainen *et al.* (2000) observed CO_2 induced increase in needle cross-sectional width after the first year of exposure, and an accumulation of starch and slight curling and swelling of the chloroplast thylakoids in the mesophyll tissue of needles of Scots pine seedlings.

The Transmission Electron Microscopy (TEM) study of leaf tissues showed a significant increase in the thickness of epidermis, size of mesophyll cells, accumulation of starch and size and number of starch granules per chloroplast in *Brassica juncea* (Upreti *et al.*, 2001). They demonstrated that the feedback inhibition did not occur because of the larger palisade and epidermal cells, which compensate the adverse water stress effect on these components. It appears that leaf anatomy reflected an optimisation strategy due to high CO_2 that allowed the loading of chloroplast with excessive oval starch grains to meet the adverse effect of drought on these components in *Brassica juncea* leaves.

Interactive effect of elevated CO_2 and drought

Virtually any factor that affects plant growth can influence its reaction to elevated CO_2 . The role of CO_2 as a chief input to plant life have greater significance when the plant is to face various stressful environments. Moderate moisture stress decreases cellular turgor, reducing expansion of leaves and other organs and stomatal conductance for water vapour and CO_2 . High CO_2 induced partial closure of stomata, slows down rates of water loss and delays the onset of stress. This may permit expansion of organs beyond that in normal CO_2 and enhance assimilation under stress. This has significant importance because stress decreased the expansion of organs more than it slows photosynthesis. The significant increase in growth with a limited water supply is in accordance with response of WUE to CO_2 .

Vu *et al.* (1987) conducted an experiment on soybean and observed that both CO₂ enrichment and drought stress reduced the total extractable Rubisco activity as expressed on chlorophyll basis. When canopy CER values and leaf water potentials were lowest, the drought stress reduced the initial RuBPcase activity by 50 per cent compared to corresponding unstressed treatments. They concluded that in soybean leaves, a non-stomatal limitation of leaf photosynthesis under moisture stress condition appears, due to a reduction of the *in vivo* activity of RuBPcase. It was observed that in *B. oleracea*, the growth promoting responses of enriched CO₂ were more pronounced at low water supply (Sritharan and Lenz, 1991).

Vassey *et al.* (1991) examined the SPS in leaves of *P. vulgaris* L. cv. Linden in plant subjected to water stress and various CO₂ and light treatments. They showed that when water was withheld causing mild water stress (-0.9 Mpa), the SPS activity was reduced to 50 per cent. One per cent CO₂ environment can reverse the effect of water stress, though the water stress was not relieved. They concluded that the effect of water stress on SPS activity was a consequence of the inhibition of photosynthesis caused by stomatal closure.

Vu *et al.* (1998) rice cv. IR-72 was grown for a season in sunlit, controlled environment chambers at 350 or 700 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ under continuous flooding (unstressed) or with water stress from panicle initiation (59 days after planting, DAP) to 76-77 DAP (when canopy net photosynthetic rate (Pn) decreased to near zero). In the unstressed plants, CO₂ enrichment increased water use efficiency (WUE) by 26 per cent and reduced evapotranspiration (ET) by 8-14 per cent. They observed that both high CO₂ and severe water stress decreased the activity and content of ribulose biphosphate carboxylase-oxygenase (rubisco) enzyme. The apparent catalytic turnover rate (Kcat) of midday fully activated Rubisco was not altered by high CO₂, but was reduced by water stress by 17-23 per cent. Chloroplasts of the high CO₂ leaves contained more and larger starch grains than those of the ambient CO₂ leaves. High CO₂ did not affect the leaf sucrose content

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of unstressed plants, while water stress reduced the leaf starch and increased the sucrose content in both CO₂ treatments. The activity of leaf sucrose phosphate synthase of unstressed plants was not affected by high CO₂, whereas that of ambient CO₂ plants was reduced 45 per cent by water stress. Reduction in ET and increases in both Pn and WUE in rice grown under high CO₂ helped to delay the adverse effects of water stress and allowed the stressed plants to assimilate CO₂ for an extra day.

The response of *Brassica juncea* cv. Pusa bold to elevated CO₂ was studied under water stress. Carbon accumulation in leaves, stem and roots was significantly higher at elevated CO₂ concentration. The water stress decreased and carbon content in these plant parts and this adverse effect was reduced by CO₂ enrichment. On the contrary nitrogen content of leaves, stem and roots was significantly reduced at elevated CO₂. Water stress caused reduction in nitrogen content in these plant parts, similar at ambient as well as elevated CO₂ concentration (Uprety and Rabha, 1999).

Ellsworth (1999) investigated leaf-level CO₂ and H₂O exchange responses and plant and soil water relations in a maturing loblolly pine (*Pinus taeda*) stand in a free-air CO₂ enrichment (FACE). According to him photosynthesis in upper canopy foliage was stimulated by 50-60 per cent by elevated CO₂. Significant photosynthetic enhancement by CO₂ was maintained over a range of conditions except during peak drought. It was concluded that large photosynthetic enhancements in the upper canopy of *P. taeda* by elevated CO₂ indicate that this maturing forest plant may have a large carbon sequestration capacity with limiting water supply.

Li *et al.* (2000) reported that wheat grain weight is a function of rate and duration of grain growth and is affected by photosynthate supply. Drought stress reduces photosynthate production because of stomatal closure. However, this might be partially overcome by an increase in air CO₂ concentration. In their studies spring wheat cv. Yecora Roja was sown on 15 December 1992 and grown at 2 CO₂ concentrations, 550 (elevated) or 370 (ambient) $\mu\text{mol mol}^{-1}$ and 2 water

treatments (well-watered, allowing 30 per cent of available water in the rooted zone to be depleted or limited water, which supplied only half as much water at each irrigation) in a Free-Air CO₂ Enrichment system in a field experiment Both elevated CO₂ and water treatments significantly influenced the grain-filling processes. Under drought stress conditions, elevated CO₂ increased grain weight in the upper and lower sections of the main stem spike by 10 and 24 per cent, respectively. In well-watered plants, final grain weight in the midsection of the main stem spike was 8 per cent higher than that measured under drought stress conditions. Grain weight increase under elevated CO₂ was due to a faster rate of grain filling. Effects of elevated CO₂ on the statistically derived duration of grain filling were inconclusive because of the confounding effect of blower-induced temperature changes on the process. The increase in grain weight of well-watered plants was due to a longer grain-filling period. Later-formed tiller spikes were more responsive to elevated CO₂ and drought stress than main stem spikes. Information from this study has helped them to understand the grain growth of wheat and provide information to establish grain growth mechanism.

Derner *et al.* (2001) reported that aboveground growth of C₄ plants (*Tallgrass prairie*, *Schizachyrium scoparium* and *Andropogon gerardii*), responds more strongly to atmospheric CO₂ concentration when soil water is limiting rather than abundant.

Wall (2001) reported that rising levels of atmospheric CO₂ affect transpiration and water absorption processes that influence total leaf water potential (PSIW) of wheat. A 200 µmol mol⁻¹ rise in atmospheric CO₂ concentration resulted in a curvilinear increase in drought stress alleviation from 0 to 77 per cent at predawn, 0 to 67 per cent at midday and 0 to 79 per cent at sunset. Hence, as the CO₂ concentration of the atmosphere rises, both drought avoidance and tolerance mechanisms will be enhanced resulting in improved water relations in wheat.

The interactive effect of atmospheric CO₂ concentration and soil-water content on grain sorghum (*Sorghum bicolor*) was reported by Wall *et al.* (2001).

The sorghum plants were exposed to ambient (control) and free-air CO₂ enrichment (FACE, ambient+200 $\mu\text{mol mol}^{-1}$), under ample (wet, 100% replacement of evapotranspiration) and reduced (dry, post planting and mid-season irrigations) water. FACE reduced seasonal average stomatal conductance (gs) by 0.17 mol (H₂O) m⁻²s⁻¹ (32 and 37% for dry and wet, respectively) compared with control; this was similar to the difference between dry and wet treatments. FACE increased net assimilation rate (A) by 4.77 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ (23 and 9% for dry and wet, respectively), whereas dry decreased A by 10.50 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ (26%) compared with wet. Total plant water potential (psiw) was 0.16 MPa (9%) and 0.04 MPa (3%) less negative in FACE than in the control treatment for dry and wet, respectively. By ameliorating the adverse effects of drought, elevated atmospheric CO₂ improved plant water status, which indirectly caused an increase in carbon gain.

Polley *et al.* (2002) reported that traits that promote rapid growth and seedling recruitment when water is plentiful might become a liability when seedlings encounter drought. They tested the hypothesis that CO₂ enrichment reinforces any trade-off between growth rate and drought tolerance by exaggerating inter specific differences in maximum relative growth rate (RGR) and survivorship of drought among seedlings of five woody legumes (*Acacia farnesiana*, *Gleditsia triacanthos*, *Leucaena leucocephala*, *Parkinsonia aculeata* and *Prosopis glandulosa*). According to them survivorship of drought at ambient CO₂ concentration was negatively related to RGR in well-watered seedlings in one of two experiments, but the relationship was weak because interspecific differences in RGR were small. There was no significant relationship among well-watered seedlings between RGR at ambient CO₂ and either the relative or absolute increase in RGR at elevated CO₂. As predicted, however, CO₂ enrichment reinforced interspecific differences in survivorship of seedlings exposed to similar rates of soil water depletion. Doubling CO₂ improved seedling survivorship of the most drought-tolerant species throughout the period of soil water depletion, but did not consistently affect survivorship of more drought-sensitive species. Midday xylem pressure potentials

of drought-treated seedlings were less negative at elevated CO_2 than at ambient CO_2 , but no other measured trait was consistently correlated with improved survivorship at high CO_2 . Carbon dioxide enrichment may not reinforce species differences in RGR, but could exaggerate interspecific differences in drought tolerance. To the extent that seedling persistence in grasslands correlates with drought survivorship, and their results indicate a positive effect of CO_2 enrichment on recruitment of woody legumes that are currently tolerant of drought.

Kang *et al.* (2002) demonstrated that the daily photosynthesis rate was increased more in wheat and cotton at high CO_2 concentration than in maize. In addition, differences were more substantial at low soil water treatment than at high soil water treatment. The daily leaf transpiration was reduced significantly in the three crops at the high CO_2 concentration. The decrease at low soil water was smaller than at high soil water. Crop biomass production response showed a pattern similar to photosynthesis, but the CO_2 -induced increase was more pronounced in root production than shoot production under all soil water treatments. Low soil water treatment led to more root biomass under high CO_2 concentration than high soil water treatment. CO_2 enrichment caused a higher leaf water use efficiency (WUE) of three crops and the increase was more significant in low than in high soil water treatment. Crop community WUE was also increased by CO_2 enrichment, but the increase in wheat and cotton was much greater than in maize. They concluded in the short-term, C_3 plants such as wheat and cotton might benefit from CO_2 enrichment especially under water shortage condition.

Does the rise in atmospheric CO_2 compensate in anyway for the decrease in C gain brought about by water deficit in plant? The literatures cited in the above paragraphs, affirmatively centre on this question. But the magnitude of this compensatory response requires further evaluation and the process needs further comprehensive characterization.

Senescence

Senescence is a highly regulated process, which is under genetic control.

In monocarpic plants, the onset of fruit development is the most important factor initiating the senescence process. In general O_2 is necessary for chlorophyll degradation (Tripi and Thimann, 1983), foliar abscission (Leopold, 1967) and for evolution of ethylene (Jackson, 1978). The importance of oxidation has been shown by the capacity of hydro-peroxide to induced membrane deterioration (Packer *et al.*, 1967), by the increased level of hydro-peroxide during senescence (Dhindsa *et al.*, 1982). Lipoxygenase (LOX, Linolate : Oxygen oxidoreductase, EC 1.13.11.12) are a class of non-heme, iron-containing dioxygenase that catalyze the oxygenation of polyunsaturated fatty acid with conjugated diene hydro-peroxide. Both lipid peroxidation and hydroperoxide promote losses of membrane integrity, protein and chlorophyll resulting yellowing (Yamuchi *et al.*, 1986 and Warda *et al.*, 1990), The hydroperoxide are key intermediates of octadecanoids signaling pathway in plants (Griffith *et al.*, 2000). It has specific role for senescence. In vitro, chlorophyll bleaching was accelerated by the presence of PUFA/LOX (Orthoetor and Dugan, 1973). These results suggested that LOX mediate lipid peroxidation might be involved in chlorophyll degradation (Zhuang *et al.*, 1994). Physiological level of the lipid hydroperoxide (LHPO) and how it is regulated in plants still unclear. This may due to their instability, and often require expensive and dedicated equipment for detection. For these reasons, the thiobarbuteric reactive substance (TBARS) estimate the amount of malondialdehyde (MDA), a secondary end product of PUFA oxidation has been used as index of general lipid peroxidation (Hodges *et al.*, 1999). Dhindsa *et al.* (1982) showed an increase in hydroperoxide during senescence of oat leaf segment and with Van Zutphen and Cornwell's (1973) observation showing that hydro-peroxidation of unsaturated fatty acid in monomolecular and bimolecular films lead initially to increase in membrane permeability and then decrease membrane stability. Packaging of broccoli under elevated CO_2 reduced O_2 , which preserves the color and improve the market quality (Barth *et al.*, 1993).

Hardy *et al.* (1978) described delaying senescence (5-10 days) by CO_2 enrichment improved photosynthesis, which lead to increase in yield in legumes.

Goudriaan and Bijlsma (1987) found that increased CO_2 enhanced photosynthesis and delayed senescence in *Vicia faba*. The beneficial effect of CO_2 on growth was maintained during water stress. The effects of additional CO_2 and water were multiplicative. Frick *et al.* (1994) reported that CO_2 enrichment delayed the onset of flowering and senescence in *Brassica*.

Li *et al.* (2000a) studied the long-term effects of elevated CO_2 concentration on the leaf senescence of *Quercus myrtifolia*. According to them the leaf senescence was delayed during this period at elevated CO_2 and delayed leaf senescence of leaves was an important consequences for leaf photosynthesis. They noted that under elevated CO_2 , the net photosynthetic rate of leaves increased significantly. In ambient CO_2 the net photosynthetic rate, 54 per cent lower than of elevated CO_2 .

Antioxidant enzymes

It has been suggested that plants grown with elevated CO_2 might be better protected from environmental stress by an increased capacity for detoxification and repair (Carlson and Bazzaz, 1982). In several herbaceous plant species antioxidative systems were induced in response to drought stress (Smirnoff, 1993). Possibly plant grown at an elevated CO_2 concentration contain increased metabolic reserves and thus, might have an enhanced metabolic flexibility to respond to oxidative stress compared with plants grown at ambient CO_2 concentrations (Schwanz *et al.*, 1996a). Production of reaction active species (ROS), including superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH), is an inevitable consequence of life in an oxygen rich environment under several stress condition (Polle *et al.*, 1990). ROS damage plant cell by oxidizing membrane lipids including photosynthetic apparatus and also damage the proteins, chlorophyll and nucleic acids (Foyer *et al.*, 1994). H_2O_2 is generated as metabolite of enzymatic reaction and during photosynthesis (Salin, 1987). The antioxidant ascorbate and glutathione participate in both enzymatic and non-enzymatic H_2O_2 degradation (Smith *et al.*, 1990). Peroxidase decompose H_2O_2 by oxidation of co-substrate such as phenolic

compounds or antioxidant (Polle *et al.*, 1990). ROS are formed within plant cell in several ways:

- (1) Photochemical production of H_2O_2 in the atmosphere from air pollution (Masuch *et al.*, 1986);
- (2) Donation of electrons directly to oxygen during photosynthesis, especially with high light or low chloroplastic CO_2 concentrations (Foyer *et al.*, 1994)
- (3) In response of stress such as drought (Smirnoff and Colombe, 1988) and
- (4) Serious nutrients imbalance (Marschner and Cakmake, 1989).

Plants have their own systems for scavenging superoxide radicals. Superoxide dismutase (SOD) catalyzes the disproportionation of O_2^- to H_2O_2 and O_2 . H_2O_2 is decomposed by catalase or ascorbate peroxidase (APX), the latter enzyme oxidizing ascorbate to monodehydroascorbate (MDA), which is then re-reduced by NAD(P)H in a reaction catalyzed by monodehydroascorbate reductase (MAR). MDA also undergoes spontaneous dismutation to ascorbate and dehydroascorbate (DA), which is re-reduced to ascorbate by the reduced form of glutathione (GSH) in a reaction catalyzed by dehydroascorbate reductase (DAR), with resultant production of the oxidized form of glutathione (GSSG). Finally, NADPH reduces GSSG again in a reaction catalyzed by glutathione reductase (GR). Thus, six antioxidative enzymes (SOD, catalase, APX, MAR, DAR, and GR) and two antioxidant substrates (ascorbate and glutathione) are involved in O_2^- -detoxification in plants (Asada and Takahashi, 1987 and Smirnoff, 1993). It has previously been observed that growth of Norway spruce for one season at an elevated CO_2 concentration resulted in decreased activities of SOD and CAT in current-year needles (Polle *et al.*, 1993). CAT activity was also reduced in tobacco grown at elevated CO_2 concentration (Havir and McHale, 1989).

Plants grown under elevated CO_2 were decreased intrinsic oxidative stress as compared to plant grown under ambient CO_2 . Because, under elevated CO_2 plant showed lower rate of photorespiration and respiration (Wullschelger *et al.*, 1994 and Polle, 1996). Since H_2O_2 product of these pathways, the necessity for

detoxification of H_2O_2 might be diminished plant growth under elevated CO_2 . They also reported that the intrinsic oxidative stress would decrease if the demand for NADPH was increased and fewer electrons were available for reduction of O_2 . Lower malondealdehyde (MDA) content was found in leaves from plant grown at elevated CO_2 (Polle *et al.*, 1996 and Polle *et al.*, 1997).

In higher plants, Cu, Zn, Fe SOD is mostly associated with the chloroplast (Salin, 1987), where they scavenge super oxide anions originating from “leakage” of photosynthetic transport chain, PSI (Mehler reaction) and reduced ferredoxin, especially under condition, which favors NADPH accumulation (Halliwell and Gutteridge, 1989). It could be expected that increasing atmospheric CO_2 enhances the demand for photo-reducing power and this could reduce risk of oxyradical formation. In this context, the release of chloroplastic SOD activity would reduce in soybean (Badani *et al.*, 1993). Water content and soluble protein of the leaves were not affected by elevated CO_2 . Both the soluble protein and water content of leaves were similar to those of well watered plants like oak and pine (Schwanz *et al.*, 1996a)

Schwanz *et al.* (1996b) investigated the effect of an enhanced CO_2 concentration alone in combination with water stress on antioxidative system on two tree species. They found elevated CO_2 significantly reduced activities of some enzymes in both pine and oak. In moisture stress plant, the activity of antioxidant enzymes in leaves decreased in at ambient CO_2 , but increased in elevated CO_2 .

According to Pritchard *et al.* (2000) constitutive antioxidative enzymatic activities may decrease in a high CO_2 world because of decrease in cellular ROS production. Significant reduction in activity were observed for SOD, POD, CAT, AOPD and GR in plant grown under elevated CO_2 condition but the up- regulation or down-regulation of plant antioxidant system depends on the extant of oxidative stress (Polle *et al.*, 1997). CO_2 and genotype interactions, however, suggested that there may be key genotypic difference in response pattern, potentially conferring differential resistance to biotic and abiotic stress, (Pritchard *et al.*, 2000).

Marabottini *et al.* (2001) reported that when oak trees (Holm oak, *Quercus ilex*) and white oak, *Q. pubescens*) exposed permanently to elevated CO₂, the activities of antioxidant enzymes tended to be lower and the ascorbate pool was larger and more in reduced form, suggesting an attenuation of the oxidative risk in the CO₂-enriched trees. In the enriched individuals of both species, the imposition of water shortage significantly increased the size of the glutathione pool and the total superoxide dismutase activity in a species-specific manner. Moreover, water-stressed trees exposed to elevated CO₂ tended to have higher catalase and ascorbate peroxidase [L-ascorbate peroxidase] activities than water-stressed control trees. Such changes may reflect the need for enhanced compensatory efforts when trees acclimated to elevated CO₂ are exposed to oxidative stress-promoting environmental factors, such as water shortage.

Most of the works reported in the aforementioned pages were based on studies carried out under temperate environment and with temperate crop and trees either in growth chamber or in controlled chamber. However, very little information is available in rising CO₂ responses on plant under tropical condition. The present study will attempt to address many questions related with plant grown under sub-tropical environment using open top chamber technology.

3. MATERIALS AND METHODS

Plant material

The response of *Brassica* species to the elevated CO₂ was studied in the present investigation using Open Top Chambers (OTCs) and Free Air CO₂ Enrichment Technology (FACE) to simulate the doubling CO₂ concentration.

Brassica is a tall herbaceous annual; growing 1-1.5 meters; stem glabrous; leaves of two types; the lower one known as the stem leaves and the upper arising on the axis of the inflorescence known as floral leaves. The floral leaves distinct from the stem leaves, either lanceolate or obtuse. Its inflorescence is long raceme, cymose when young; the flowers are hermaphrodite, cruciform; ovary superior, bicarpellary, parietal placentation, ovules many; fruit siliquae. Seeds are cotyledonous, contains oil (Dutta, 1976). It has tap root system, which can grow very deep in the soil particularly in sandy soil under dryland agriculture, where most of the oilseed is grown in India (Labana *et al.*, 1992).

Brassica cultivars viz. 'Pusa Gold' (*Brassica campestris*, 2n=20aa) and 'RH-30' (*Brassica juncea* 2n=36aabb) were selected for this investigation due to variation in their response to moisture stress and difference in their genomic configuration (Fig. 8). The *Brassica campestris* was susceptible to drought compared to *Brassica juncea* (Upreti *et al.*, 1995). Both the cultivars are commercially important and widely cultivated under rainfed condition. (Saran and Giri, 1987).

The leaf blade of 'Pusa Gold' (*Brassica campestris*) is non hairy and grasp the stem completely and seeds are yellowish, whereas, in 'RH-30' (*Brassica juncea*) it is hairy and does not reach the stem and seeds are brownish in colour.

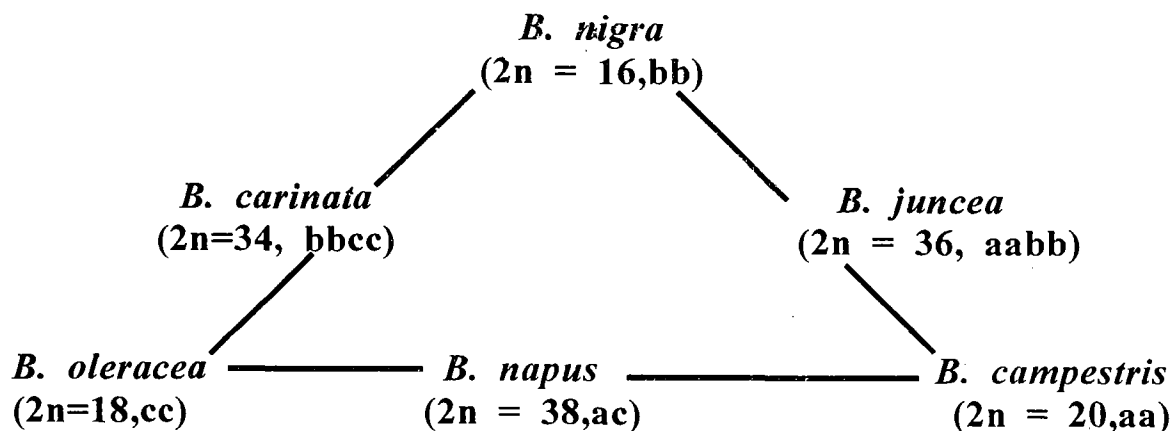


Fig. 8 Cytogenetical relationships between species of *Brassica* (U 1935)

Plant culture

These experiments were conducted in the field of Plant Physiology division for two consecutive Rabi seasons (2001 and 2002) under the “National Fellow Project”, ICAR, and New Delhi, India. Field was prepared by four ploughings and planking. The land within the chambers was prepared by hoeing. Farmyard manure (FYM) was applied @ 5 tons/ha during the time of field preparation. Pure, healthy seed were sown on petridishes and germination test was done before sowing in the field.

The plant spacing, fertilization and other cultural practices were followed according to recommended package and practice. Forty-five cm inter row spacing and 20 cm between the plants were maintained. Nitrogen, phosphorus and potassium fertilizers were applied at the rate of 30+30: 60: 40 kg per hectare. Half of the nitrogen was applied as basal dose and another half at the time of flowering (Dey *et al.*, 1978). The plants were sprayed with metasystox 25EC to control aphid. The regular hand weeding was done to remove the weeds.

Moisture stress treatment:

Moisture stress treatment was given by restricting irrigation at following stages of growth:

- (a) Vegetative (25 DAS),

- b) Flower bud appearance (45 DAS),
- c) 50% flowering (60 DAS) and
- d) Post flowering (75 DAS)

Soil moisture content was measured using gravimetric method (Dastane, 1972). Triplicate soil samples were augured out from an average depth of 15-20 cm and collected in aluminum containers. The container plus fresh soil sample was weighed. Soil was dried in an oven at 105 °C. Drying was allowed to continue until a constant weight was attained. The weight of container plus dry soil was taken. The difference between the fresh weight and dry weight of soil determined the moisture content in the soil, which was expressed as percentage.

$$W = \frac{W_1 - W_2}{(W_2 - c)} \times 100$$

Where, W= Soil moisture per cent, W_1 = Weight of moist soil + container, W_2 = Weight of dry soil + container and c= weight of container

The average moisture content of soil ranging from 23-25 per cent under irrigated condition and it was maintained between 8-10 per cent under moisture stress condition during crop growth period. (Fig.9)

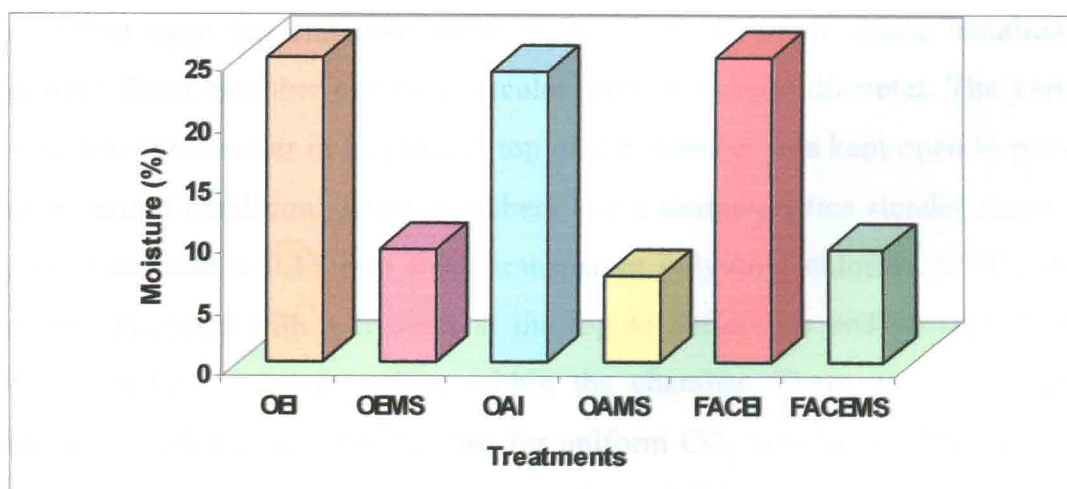


Fig. 9. Average soil moisture content in OTC and FACE

CO₂ enrichment technology

To simulate the doubling CO₂ concentration, Open Top Chambers (OTCs) and Free-Air CO₂ Enrichment (FACE) technologies were used. Two sets of experiment were conducted in CO₂ enriched and ambient atmosphere, as below:

1. Plants grown in OTC

- i. Ambient atmospheric condition
- ii. Elevated CO₂ concentration (CO₂ level $600 \pm 15 \mu\text{mol mol}^{-1}$)

2. Plants grown in FACE

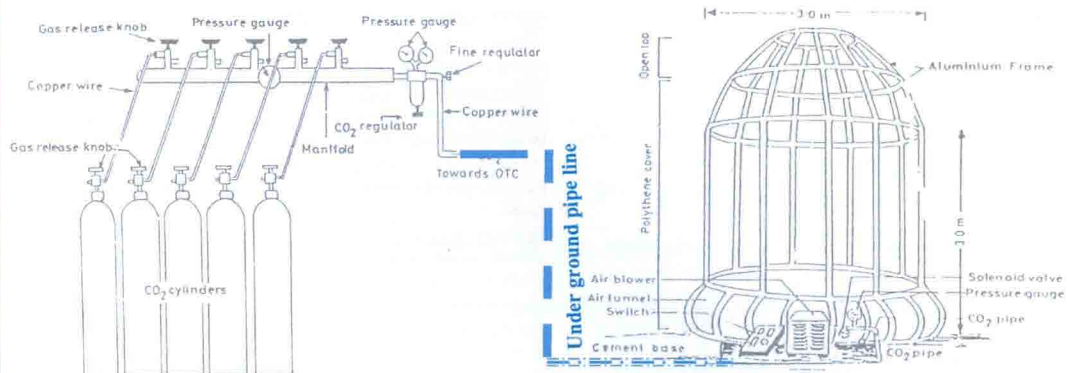
- i. FACE ring (CO₂ level $550 \pm 15 \mu\text{mol mol}^{-1}$)
- ii. Ambient air: which was from 60 meter away from FACE ring.

Open top chambers (OTCs)

CO₂ enrichment technology comprised five sub-systems (Fig. 10).

- (a) Supply of pure and high concentration of CO₂,
- (b) System of valves, regulators and flow meters,
- (c) Chambers or area to be CO₂ controlled,
- (d) Appropriate gas analyzer with feed back control, and
- (e) Computed data acquisition and programming.

These open top chambers were made of an aluminum frame installed on the ground. Each chamber covers a circular area of 3-meter diameter. The vertical sidewall was three meter in height and top of the chamber was kept open to provide the near natural condition. These chambers had a characteristics slender shape and were covered with a 0.15 mm thick transparent polyvinyl chloride (PVC) sheet. Chambers equipped with a frustum at the top to deflect air and prevent dilution of the desired CO₂ concentrations within the chamber. There was a cylindrical double walled plenum around the base for uniform CO₂ circulation. The inner side of the plenum was having numerous gas outlets of different sizes, the small being nearest to the gas inlet pipe. Commercial CO₂ was added in the upstream of the frame to ensure adequate mixing and to create the desired concentration. Blowers



Gas cylinders

CO₂ regulating systems

- A = Supply of pure and high concentration of CO₂
 B = System valves, regulators and flow meters
 C = Chambers or area to be CO₂ controlled
 D = Appropriate gas analyzer with feed back control
 E = Computed data acquisition

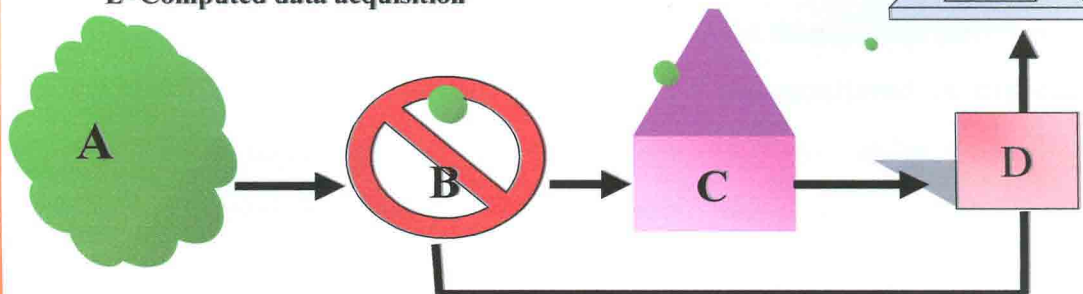


Fig 10 Skeleton CO₂ regulating systems



Open Top Chambers with *Brassica* crop.

were used to distribute CO₂ enriched air uniformly throughout the plant canopy. The CO₂ gas was supplied to the chamber from gas cylinders using a manifold, gas regulators and pressure gauge pipeline. Five source gas cylinders of 45 kg capacity each were fitted in a row with thick copper outlet pipe (Fig. 11). CO₂ gas was released from the cylinders to the chamber through a manifold and underground pipe and injected into the chamber along with the ambient air using a blower. The air blower fitted at the base of the chamber was of 12 inch in diameter, which thoroughly mixed the CO₂ with atmospheric air and blowed the gas mixer to the chamber tunnel. The blower also helped in maintaining the inside air temperature closer to that of outside ambient atmosphere. The relative photosynthetic photon flux density (PPFD) in the chambers was maintained about 95 per cent of full sunlight by frequent and gentle washing of the transparent polythene sheet. The daily observation on temperature and RH were monitored in the chambers and recorded in thermo hygrograph (Lawrence and Mayo, India model: w/1712/z). Weekly changes in chart paper of thermo hygrograph were done throughout the crop growth period in both the crop seasons of 2001 and 2002. The diurnal variation in temperature and CO₂ inside the chamber was recorded (Fig. 12, 13). Chamber air temperature was slightly higher compared to field plots and the FACE ring. The hourly value of light intensity inside the chambers were also recorded (Fig. 14):

The concentration of CO₂ in the OTC was regulated by three-step operation:

- (a) Standardizing the gas release from the source to the manifold with the help of a pressure gauge
- (b) Opening two successive finer metered valves for controlled release of gas from manifold to the under ground pipe, and
- (c) Adjusting the solenoid valve at the end of the pipe with the help of pressure meter.
- (d) The monitoring of CO₂ concentration was done by Infra Red gas analyzer (IRGA) by drawing air from different positions of crop canopy. Before

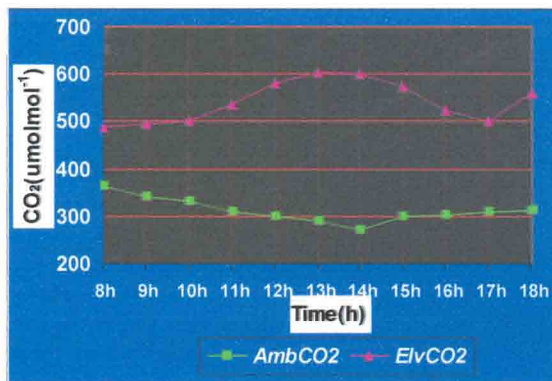


Fig. 11 Diurnal variation of CO₂ concentration within the OTC



Fig. 12. Diurnal variation of temperature within the OTC

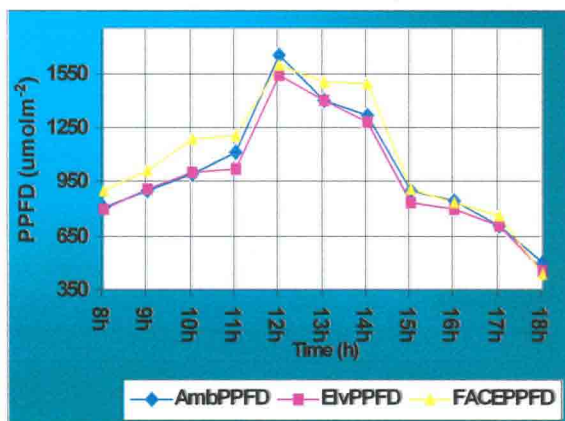


Fig. 13 Diurnal variation of photosynthetic photon flux density (PPFD) within the OTC

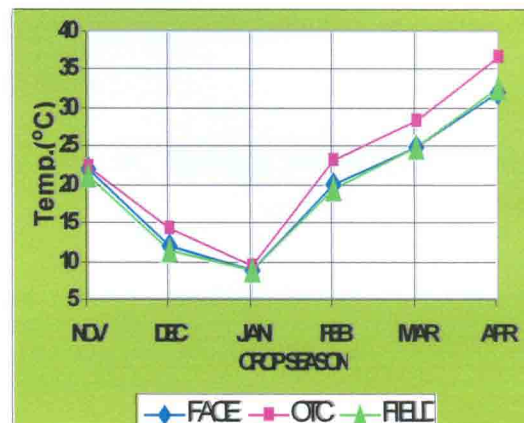
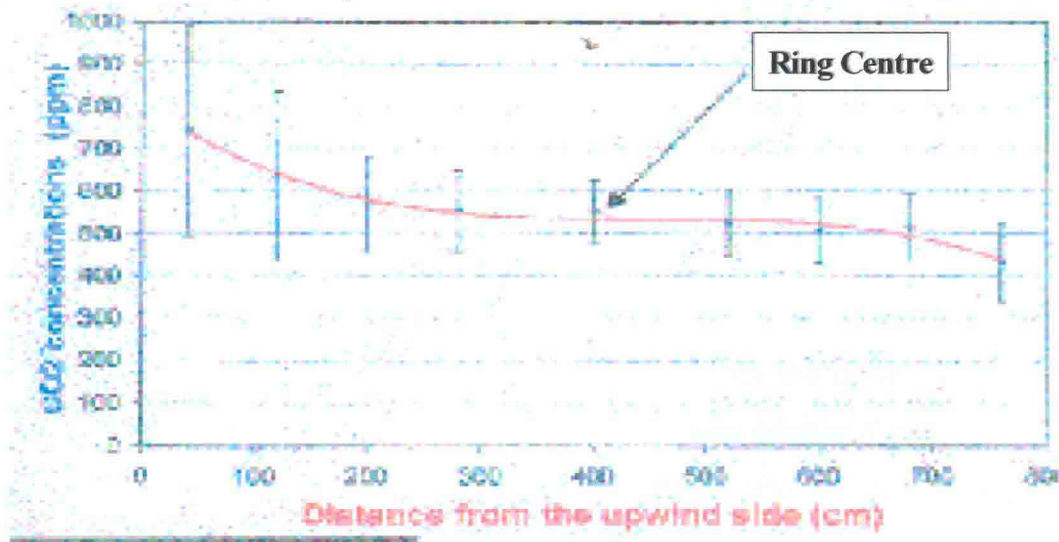


Fig. 14 Monthly average temperature in FACE, OTC and Field



measurement, of the CO₂ concentration in the atmosphere, the IRGA was calibrated in absolute mode (Uprety *et al.*, 1995, 1998)

Free-air CO₂ enrichment (FACE) Technology

The Free Air CO₂ Enrichment (FACE) technology was based on the principle of injecting additional CO₂ gas in open field suitably so as to attain a predetermined elevated level of gas concentration with uniform distribution in the field under varying meteorological conditions of wind, temperature and humidity. The main components of a FACE facility were:

- i. CO₂ gas storage and distribution system.
- ii. The FACE ring, generally known as plenum, through which CO₂ enriched air was injected into the open field.
- iii. Different sensors and actuators used for monitoring the environment and controlling the operations.
- iv. An electronic system for controlling these various operations.

System design:

The Mid-FACE ring was octagonal in shape having a diameter of 8 m as shown in the block schematic diagram (Fig. 15). The eight arms of the FACE ring were made of rigid PVC pipes of 200 mm diameter and wall thickness of 4.5 mm. Each arm of the octagon was fitted with a centrifugal air blower at one end for blowing air into the pipe and was closed at the other end. Four rows of holes (4 mm diameter) were drilled on each arm for the outlet of CO₂ enriched air. The CO₂ was injected at the input of each arm blower for pre-mixing with air within the pipe before its injection in the fields. The air blower pressure and holes on the pipe were arranged in such a way that the air outlet from the holes along the whole length of pipe remained at uniform pressure. All the eight arms of the octagon had independent supply of CO₂ controlled by 8 on/off valves. A common computer controlled proportional integral differential (PID) valve controlled the quantity of CO₂ to be released into the arms.

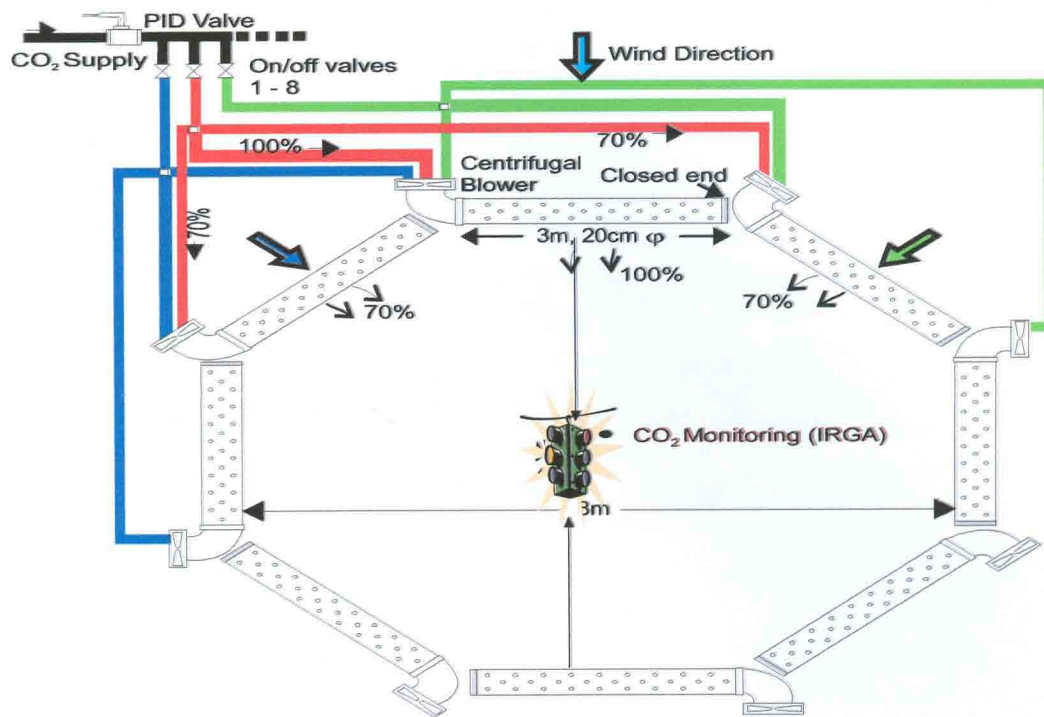


Fig. 15 MID FACE Ring for South Asia.

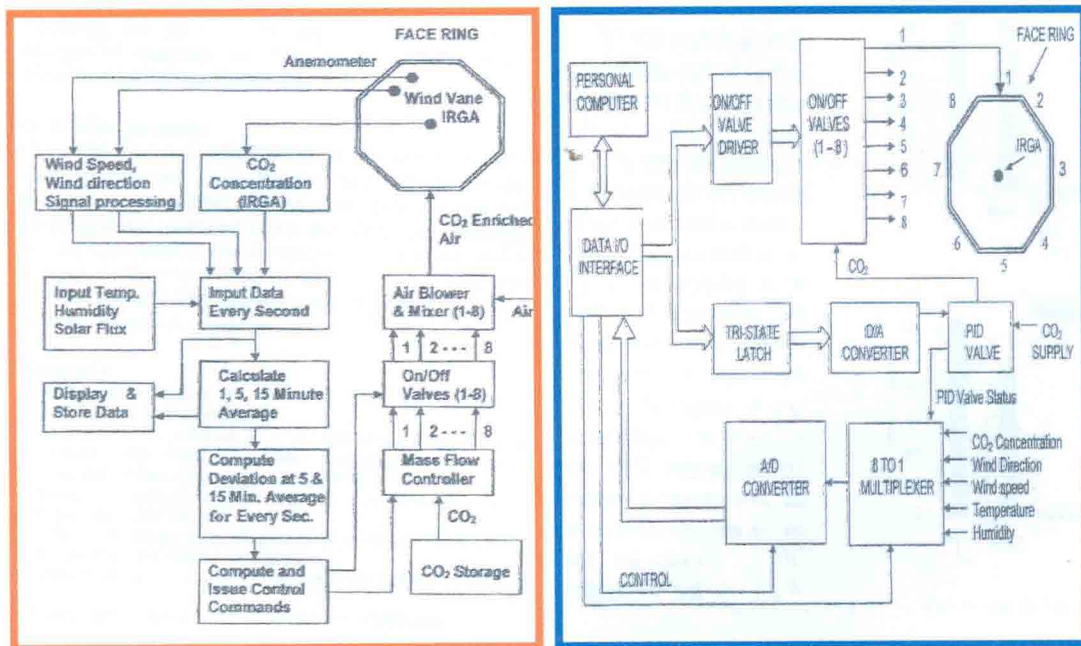
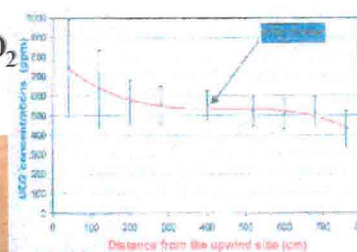


Fig. 16 Block schematic of the system controller for Mid-FACE **Fig. 17 Flow chart for control sequence**



computer system

Diurnal variation of CO_2 within FACE during crop season



IRGA



Vent pipe

**Crop in
Mid-FACE ring**



PID valves



Brassica crop in FACE ring

The CO_2 was released at a time into the field from 3 adjacent arms of the FACE ring. There were three nozzles for CO_2 injection for each arm. Depending on the downward wind direction, the central arm got CO_2 from the nozzle having 1.5 mm diameter and adjacent arms got supply from nozzles having 1.0 mm diameter. As shown in Fig. 15, when the downward wind direction was towards the arm 2 side, the on/off valve no. 2 was switched on and CO_2 was released from the arms 1,2 and 3. The CO_2 was injected into the arm 2 through the nozzle of 1.5 mm diameter and into the arms 1 and 3 through the nozzle of 1.0 mm diameter to take care of the effect of tilt of adjacent arms with wind direction. Thus, at any given time, for any wind direction, CO_2 was always released only through 3 arms of the FACE ring per plenum. The CO_2 concentration was measured at the center of the field with the help of an Infra-Red Gas Analyzer (IRGA WMA-2). Sampling of gas was done continuously through a thin polythene tubing (4.3 mm ID) with the inlet height adjusted at about 5-25 cm above the crop canopy. The output from IRGA was the DC voltage in the range 0.5 V corresponding to 0-2000 ppm of CO_2 .

The concentration of CO_2 in open field not only depended on the quantity of CO_2 enriched air released from the plenum, but also on the gas dispersion characteristics controlled by the meteorological factors such as wind speed, wind direction, humidity and temperature within the plenum. The sensors for wind speed (anemometer), wind direction (wind vane) and temperature were located near the center of the FACE ring at plant canopy height. Their output signals were continuously measured in real time by the system controller. The output from the wind speed sensor was a DC voltage in the range of 0-5 V corresponding to wind velocity of 0-33 m/s and output from the wind vane was also a DC voltage in the range of 0-5 V corresponding to wind direction of 0-359⁰ with reference to the north.

The PID valve controlled the quantity of CO_2 to be released into plenum arms, which depends on the control voltage, applied to it (0-10 V). To evaluate

the proper operation of the PID valve, a feedback signal (from PID valve) was also monitored to know the actual status of the valve. This signal was in the range of 0-5 V corresponding to the fully closed and fully open conditions of the PID valve.

The fumigation of CO₂ gas into the field from the plenum was made at the crop canopy height. The height of plenum could be adjusted from time to time to match with the height of the crop canopy by resting the plenum on height-adjustable stands. Plenum could be raised up to 5 feet from the ground level in steps of 2 inches. Two banks of 25 cylinders in parallel were used for CO₂ storage and dissemination under constant pressure. Normally, one bank of cylinders supplied CO₂ to the ring. The second bank was switched on when CO₂ got exhausted in the 1st bank. A buffer tank was installed after the cylinder bank, which took care of the pressure variations in the supply line due to decreasing level of CO₂ in the cylinders during Mid-FACE operation.

Control system

The control process involved monitoring of CO₂ concentration and meteorological parameters inside the FACE ring along with the issue of control signals to a number of devices for their proper operation. A PC- based system controller was designed to control all such operations of the FACE facility. The block schematic of a PC-based system controller was shown in Fig.16. The system had been designed to measure 8 analog input signals in time-multiplexed mode. They were then digitized using a 10-bit analog to digital converter, which was connected, to the parallel port (LPT1) of PC. Thus, digitized signals entered the PC through this parallel port. The control signals required for the on/off valves (0 V-off, 5 V-on) of 8 arms of plenum were generated by the PC in the 8-bit digital form and sent out through a data I/O card. As the on/off valves operate at 24 V and require a current of 1 amp, the digital data were converted into the required analog form using suitable interface, capable of supplying the required voltage and current to the on/off valves. Similarly, the signal for controlling the

PID valve was issued by the PC in the form of an 8-bit digital word sent out through another port of the data I/O card. The 8-bit digital to analog converter converted the digital data in to analog form of 0 to 10 V required for the proper operation of PID valve. The operations of all the interfaces such as A/D converter, D/A converter, multiplexer and latches, etc. were synchronized under the control of PC.

Apart from maintaining the CO₂ concentration at the target value, the system controller was also used to record all the data, make computations and display the data in suitable tabular and graphic format. The data were recorded in the form of files.

Operation sequence

The operational sequence of the Mid-FACE facility was shown in the flow chart (Fig. 17). The data from the meteorological sensors (wind speed and wind direction) along with the CO₂ concentration were measured in every one-second by the control system. Then, 1, 5 and 15 min averages, along with their deviations from the instantaneous (every 1 second) values, were computed. Based on these inputs, the control system, through a specially developed PID algorithm, controlled the flow rate of CO₂ gas in the plenum with the help of a PID controller valve. The direction of wind decides which on/off valve was to be opened to release CO₂. In case the wind velocity was less than 0.5 m/s (which is taken as the case of calm air), the valves were opened in a cyclic order as no dispersion of CO₂ occurs in such conditions. Thus, a predetermined constant level of enriched CO₂ was maintained at the center of plenum.

Growth parameters:

Following growth observations were recorded at vegetative, flower bud initiation, flowering, post-flowering and harvesting. All the observations were taken using four replicates for each treatment.

Plant height

Height of plant was measured with the help of a meter scale from the base of plant to the apex of main axis and was recorded in centimeters (cms).

Root length

Roots were dug out at different stages of growth according to method of Sirohi *et al.* (1978). Soil was separated from roots by gentle washing. Roots were further washed over a fine sieve. The length of the tap root was measured from root/ shoot demarcation point to root tip by metallic measuring tape.

Root-Shoot ratio

The allometry of root growth to top growth was expressed by root: shoot ratio. Root : shoot ratio was calculated from the data on root and shoot length (cm) at different stages of growth.

Root volume

Root volume was determined by water displacement method used by Raja and Bishnoi (1990) and was expressed as CC. After measuring the root length, the root of each treatment submerged in 200-500 CC water measuring cylinders, the water displaced from cylinders measured by another measuring cylinder and this measurement determined the root volume.

Number of secondary root

The number of secondary roots per plant were counted from each treatment.

Number of branch

The primary, secondary, tertiary and total number of branches per plant from each treatment were recorded at the time of harvesting of the crop.

Number of Leaf

The number of leaves per plant was recorded at vegetative, flower bud

initiation, flowering and post flowering stages of growth. Physiologically mature and active leaves were considered for counting.

Leaf area

The leaves were separated from the stem and cleaned with tissue paper. The area of fresh green leaves for each treatment was measured using leaf area meter (Model. LICOR 3000, USA) and was expressed in cm² plant⁻¹.

Leaf area index (LAI) and Leaf area duration (LAD):

Leaf area index (LAI) and leaf area duration (LAD) were calculated at vegetative, flower bud-initiation, flowering and post-flowering stages using formulae as suggested by Evans (1972).

Leaf area index expresses the ratio of leaf surface (one side only) to the ground area occupied by the plant.

$$LAI = \frac{\text{Total leaf area}}{\text{Ground area}}$$

Leaf area duration was measured as leaf area index sustained per unit of time and calculated by Gardener *et al.*, (1985)'s equation.

$$LAD = LAI \times M,$$

Where, M= Number of days (crop growth period).

Plant biomass

Plants were separated into root, stem, leaves and siliquae at different stages of growth. Samples were dried in an oven at 80°C till constant weight. Dry weight of components of plant parts was measured subsequently and growth analysis was done (Gardener *et al.*, 1985.)

The dry matter partitioning to different parts of the plant i.e. leaf, stem, root, shoot, and pods was studied according to method of Thurling (1974).

Leaf Growth Analysis:

Specific leaf area (SLA)

Specific leaf area was calculated as leaf area per unit of drymatter and was expressed as cm^2g^{-1} .

$$\text{SLA} = [(A_2/LW_2) + (A_1/LW_1)]/2$$

Where, A_1 and A_2 were leaf area and LW_1 and LW_2 were dry weight of leaf for time t_1 and t_2 respectively.

Specific Leaf Weight (SLW)

SLW denote leaf dry weight per unit leaf area and was expressed as g cm^{-2} .

$$\text{SLW} = [LW_2/A_2 + LW_1/A_1]/2$$

Where, A_1 and A_2 were leaf area and LW_1 and LW_2 were dry weight of leaf for time t_1 and t_2 respectively.

Leaf Weight Ratio (LWR)

It is a measure of dry weight of leaf per unit total biomass and expressed as $\text{g g}^{-1}\text{day}^{-1}$. It indicates the biomass accumulation in leaf in comparison to other plant parts.

$$\text{LWR} = (LW_2/M_2)/(LW_1/M_1)$$

Where, LW_1 and LW_2 were dry weight of leaf and M_1 and M_2 were total dry weight of the plant for time t_1 and t_2 respectively.

Leaf area ratio (LAR)

LAR is a measurement of leaf area per unit total biomass (whole plant). It was expressed as cm^2g^{-1}

$$\text{LAR} = [(A_2/W_2) + (A_1/W_1)]/2 \text{ CM}^2\text{G}^{-1}$$

LAR expresses the ratio between the area of leaf lamina or photosynthesizing tissue and total respiring plant tissue.

Where, W_1 and W_2 were total dry weight of plant and A_1 and A_2 were leaf area for time t_1 and t_2 , respectively

Functional growth analysis:

Functional growth analysis was done by using the formulae as suggested by Radford (1967).

Net Assimilation Rate (NAR)

It is the measurement of average photosynthetic efficiency and calculation was based on increase in dry weight of plant per unit leaf area per unit time and expressed as $\text{gcm}^{-2}\text{day}^{-1}$. NAR was calculated using following formula.

$$\text{NAR} = (W_2 - W_1 / A_2 - A_1) (\ln A_2 - \ln A_1) / t_2 - t_1$$

Where, W_1 and W_2 were total dry weight of plant and A_1 and A_2 were leaf area for time t_1 and t_2 , respectively.

Relative Growth Rate (RGR)

It is a measurement of dry weight increase in a time interval in relation to initial weight. It was calculated using following formula and expressed as $\text{g}^{-1}\text{day}^{-1}$

$$\text{RGR} = \ln W_2 - \ln W_1 / t_2 - t_1$$

Where, W_1 and W_2 were total dry weight of plant and A_1 and A_2 were leaf area for time t_1 and t_2 respectively, $\ln$ = Natural logarithm base (2.71828).

Crop Growth Rate (CGR)

CGR was measured by harvesting (sampling) a crop community at frequent intervals and calculating the increase in dry weight from one sample to the next. It represented dry weight gained per unit area of crop in a unit time and expressed as $\text{gm}^{-2}\text{day}^{-1}$

$$\text{CGR} = W_2 - W_1 / (t_2 - t_1) \text{ GA}$$

Where, W_1 and W_2 were total dry weight of plant and A_1 and A_2 were leaf area for time t_1 and t_2 , respectively and GA was ground area.

Ontogeny parameters:

Phenological data were recorded as days to initiation of leaves, flower and siliquae. Data on days to flower bud initiation, flowering, siliquae formation and physiological maturity were recorded according to the method of Roy and Paul *et al.* (1991); Setia *et al.* (1995). The vegetative, reproductive, seed filling and crop growth durations were calculated.

Sink capacity:

Sink capacity is the maximum volume available for the accumulation of photosynthates. Sink capacity was calculated from numbers of pods per meter square, seed per pod and individual seed weight using formula as suggested by Thurling (1992).

Sink capacity = Pods per unit area x seeds per pod x individual seed weight

Harvest index (HI)

Plants with roots were collected at harvest. Siliquae were separated and thrashed. Seeds were cleaned and weight (economic yield). The whole plant dry weight was measured as biological yield. The harvest index was calculated as the ratio of economic yield to total biological yield and was expressed in percentage (Gardener *et al.*, 1985).

$$HI (\%) = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

Yield components:

Following yield components were recorded at final harvest:

(a) Total number of pod per plant, (b). Number of seeds per pod, (c). Pod dry-weight per plant, (d). Seed- husk ratio and (e). Seed yield (g m^{-2})

Seed quality:

Harvested seeds from each treatment were cleaned thoroughly and ground (grain weight was recorded from non ground seeds). Ground seed samples were passed through 40-mesh sieve and following quality related parameters were studied.

- i. **Grain weight** : (1000-grain weight was recorded for each treatment).
- ii. **Nitrogen** : (Kjeltec Auto 1028 analyzer).
- iii. **Protein** : (Protein was calculated by multiplying the nitrogen value with factor 6.25).
- iv. **Sugars** : (Reducing sugar, non-reducing, total sugar).
- v. **Starch** : Anthorone mehod.
- vi. **Carbon content** : (Walkley and Black, 1934).
- vii. **Oil content** : (Oil content of seeds was estimated by free induction decay method for proton pulse and NMR as reported by Tiwari *et al.* (1974) using 16 MHz pulsed NMR spectrometer).
- viii. **Crude fiber** : Crude fiber content of seeds was estimated according to the method of Thimmaiah (1999).
- ix. **Fatty acid analysis** : Analysis of fatty acid was carried by gas-liquid chromatography (Chemito GC-8510, Chemito Instrument, India) following the method of Morission and Smith (1964). This method based on the principle that fatty acid are first converted into a volatile form usually methyl ester. The ester of fatty acid were then identified by comparing with a set of standard acid ester and quantified.

Reagent

- (a) Sodium methoxide (0.5N)
- (b) Methanol AR

- (c) Hexane, 40 °C-60 °C.
- (d) Sodium sulphate (anhydrous).

GLC condition

- i. **Column:** 10% DEGS on Chromosorb, ii. **Detector:** FID, iii. **Carrier gas:** Nitrogen with a flow rate of 40-50 ml/min, iv. **Column/oven temperature:** 170-200 °C, v. **Detector temperature:** 230 °C, vi. **Injector port temperature:** 230 °C, vii. **Recorder speed:** 1cm/hr.

Preparation of methyl ester

Seeds (0.1 g) of each treatment were ground with 4 ml of 5.0 N sodium methoxide. It was transferred to air tight screw capped 15 ml vial and kept on boiling water bath for 10-15 min. Vials were cooled at room temperature. The sample was again heated for 5 minutes by adding 2 drops BF₃-methanol and then cooled and 2 ml of hexane was added with vigorous shaking. The hexane layer was separated out after few minutes.

Fatty acid analysis

Two micro liter aliquot of the hexane layer containing methyl ester was taken and injected into preconditioned gas chromatograph. Retention time was measured and the individual fatty acid was identified by comparing with the retention time of standard methyl ester. The area of each peak was calculated by measuring peak height and width at half height. Computing total peak area for the sample, percent area under each peak was calculated, which gave percent of respective fatty acid.

Gas exchange parameters

The measurement of CO₂ gas in the atmosphere was done by Infra red gas analyzer in a absolute mode.

Fully expanded uppermost leaf of main shoot was used for measuring the gas exchange parameters at different stages of growth. The measurement was done

on clear sunny days between 10.00 to 12.00 hrs, when photosynthetically active radiation (PAR) ranged between 900-1500 $\mu\text{E m}^{-2}\text{s}^{-1}$.

Photosynthesis

The rate of photosynthesis was measured on leaves using a portable Infrared Gas Analyzer (IRGA, LI-COR-6200, Lincoln, Nebraska, USA). Infrared gas analysis of CO_2 is the method of determining CO_2 concentration for the measurement of photosynthesis and respiratory rates of plants.

Operation

The target leaf inserted in the leaf chamber facing the sun. The vapor pressure was monitored on the display board after inserting the leaf, and flow rate was adjusted till a constant rate was obtained. The incident photon flux density was monitored from the quantum sensor mounted on the leaf chambers. Assimilation by the enclosed leaf area caused a depletion of CO_2 concentration inside the chamber and this depletion was measured for three consecutive times of 10 seconds each. Based on the CO_2 depletion and other factors such as temperature, pressure and volume of the enclosure, the rate of photosynthesis was computed by the inbuilt microcomputer of the IRGA, which was expressed in $\mu\text{mol m}^{-2}\text{s}^{-1}$.

The expression used in calculating photosynthesis in IRGA is:

$$A = \frac{F_d e C}{100 SP} - \frac{P G V_t}{8.314 (T_a + 273) S} - \frac{\partial C}{\partial t} - \text{CEG}$$

T_a = Chamber air temperature (C), C = CO_2 concentration (μLL^{-1}) F_d = Flow through desiccant ($\mu\text{mol s}^{-1}$), e = vapor pressure in chamber (mb), $\partial C / \partial t$ = Rate of CO_2 Change ($\mu\text{LL}^{-1}\text{s}^{-1}$), E = Transpiration rate ($\text{mol}^{-2}\text{s}^{-1}$), A = Photosynthetic rate ($\mu\text{mol m}^{-2}\text{s}^{-1}$), P = Atmospheric pressure (mb), S = Leaf area (cm^2), V_t = Total volume (cm^3).

Respiration

The respiration was measured following the method of Anthor (1994) using IRGA (LICOR-6200). The leaf chamber with the target leaf was covered for 10

minutes to avoid any penetration of radiant energy. The rate of respiration in terms of efflux CO_2 concentration was recorded and expressed as $\mu\text{mol m}^{-2}\text{s}^{-1}$.

Stomatal conductance

The stomatal conductance was measured following the method of Centritto *et al.* (1999) using IRGA (LICOR-6200). The stomatal conductance was expressed as $\mu\text{mol m}^{-2}\text{s}^{-1}$.

Internal CO_2 concentration

The intercellular concentration (C_i) of CO_2 was calculated from the measured assimilation rate and stomatal conductance. The internal CO_2 concentration was expressed in $\mu\text{mol m}^{-2}\text{s}^{-1}$.

$$C_i = \frac{[g_{tc} - E/2] C_a - A}{[g_{tc} - E/2]}$$

g_{tc} is the total conductance to CO_2 , $1/g_{tc} = 1.6/g_{sw} + 1.37/g_{bw}$, 1.37 is the ratio of diffusivities of CO_2 and water vapor in the boundary layer, 1.6 is the ratio of diffusivities of CO_2 and water vapor in air.

Water relation parameters

Uppermost fully expanded fresh leaves of main stem were collected at 10 a.m. to study the water relation parameters at different stages of growth. Four replications were taken from each treatment.

Relative water content (RWC) content of leaves

Twenty discs were taken from the leaves of each treatment and weighed. Leaf discs were submerged in water in a petridish for four hours. They were blotted and their saturated weight was measured. Discs were dried at 80°C till constant weight. Relative water content (RWC) was calculated by following equation (Weatherly and Barrs, 1962) and was expressed as percentage.

$$\text{RWC} = \frac{(\text{Fresh weight} - \text{Dry weight})}{(\text{Saturated weight} - \text{Dry weight})} \times 100$$

Leaf water potential (Ψ_p)

The water potential (Ψ_p) of leaf was measured by PMS pressure chamber (Corvallis, Oregon, USA) as reported by Scholander *et al.* (1965). The fresh leaf was covered with kilnflim to prevent the moisture loss and immediately excised with a single cut using sharp razor. The leaf was placed in the chamber with the cut end of the petiole protruding out from the rubber sleeve. The chamber was locked and then pressure was applied on the leaf at a rate of 0.2 bar per second from nitrogen gas cylinder till a drop of sap oozed out from the cut surface. Further rise of pressure was stopped and the water potential was recorded directly from gauge and expressed in MPa (Scholander *et al.*, 1965).

Osmotic potential (Ψ_s)

The fresh leaves were placed in aluminum foils and stored in liquid nitrogen for over night. Sap was extracted by squeezing leaf samples. The extracted sap was used as osmol (M). The concentration of osmol (M) was determined by vapor pressure osmometer (Model 5500, Wescor, Inc, USA). The osmotic potential of samples was determined by following formula as suggested by Wyne Jones and Graham (1983) and was expressed in MPa.

$$(\Psi_s) = M \times T \times 0.00832$$

Where, M and T denote the concentration (osmol) and absolute temperature of the sample, respectively.

Proton spin-lattice relaxation (T_1)

Fully expanded fresh leaf (6th node from the top) of main stem were collected at full sunlight (before 1200 pm, solar PPFD~1500 $\mu\text{mol m}^{-2}\text{s}^{-1}$) for study of intercellular water movement by Nuclear Magnetic Resonance (NMR) technology. NMR offers non-destructive and non-invasive method for characterizing tissue water status (Mathur-De Vre 1979). The samples were rolled into a cylinder (2 cm height) to fit tightly into a 1.8 cm diameter NMR sample tube for measurement of T_1 .

Proton spin-lattice relaxation (T_1) values were measured by 90° - τ - 90° pulse sequence (Farrar and Becker 1971) using Bruker minispec pc-20 spectrometer (20 MHz) at 25°C temperature. The non-exponential magnetization was resolved by semi-logarithmic graphic method (Tewari et al., 1986). The reactive magnitude of magnetization and corresponding relaxation time of each fraction were calculated using equation suggested by Colire *et al.* (1988).

$$M(t) = M_0 (1 - \sum_i P_i e^{-t/T_1^i})$$

Where, $M(t)$ = longitudinal magnetization at any time 't', M_0 = equilibrium magnetization, P_i = is the fraction of total spin population present at any time in the i^{th} phase. The relaxation rate ($R_1 = 1/T_1$) was calculated from the measured mean value of T_1 .

Anatomical studies:

Sampling

The stem at 7th internodes from base of main stem, root from 4 cm behind the tip of main root and fully expanded leaf (6th node from the top) of main stem were collected. The anatomical studies of root, stem and leaf were done using light microscopy (Setia *et al.*, 1997)

Leaf anatomy

For the measurement of leaf thickness, leaf segment of each treatment was initially placed in fixative FFA, comprising of formalin: acetic acid: ethylalcohol: water (10 : 5 : 50 : 35 V/V). It was then dehydrated in ethyl-butyl alcohol series and embedded in paraffin wax. Cross section was made on ultra-microtome and stained with safranin (0.5% w/v) in 50%, (v/v) alcohol. The thickness of palisade, mesophyll and combined epidermal layer were measured in terms of micro meter (μm) (Rahim and Fordham, 1991).

Root and stem anatomy

To study the stem and root anatomy, 5 mm thick stem and root section

were cut and fixed in the fixative solution (FFA) and then dehydrated in series of alcohol dilutions (70 to 100%), treated further with xylene and embedded in paraffin wax as suggested by Jensen (1962). The xylem and phloem tissues were characterized after staining with safranin. Calibration of microscope was done with the help of stage and ocular micrometer and structural parameters were determined under microscope (100x and 60x magnification) (Murti *et al.*, 2001).

Stomatal Characters:

Leaf stomata

Fully expanded leaf samples at flowering stage were collected in polythene bag. Clear nail polish was applied to both leaf surfaces and allowed to dry for approximately five minutes. Double sided cellophane tape was used to peel dried impression from the leaves. 5 cm long section of the impression at the tip, center and base positions of each leaf surface were mounted on microscope slides, and cover slip was placed. Stomata of each surface were counted in 1.85-mm² microscopic field (100x magnification) on both adaxial and abaxial surface (Cohen *et al.*, 1982).

Siliquae stomata

Siliquae at 36 days after flowering (DAF) from middle position (13th, 14th and 16th number) of main inflorescence collected from each treatment. The tip and base portion of siliquae was removed and placed in a 100 ml beaker containing 5 per cent CuSO₄ and boiled on hot plate. The sample material was cooled under running tap water and 1 per cent HCl was added and kept for 20 min. Samples were re-washed in distilled water and impression was removed by hand peeling. The impression was mounted on slide for stomatal studies as method described by Cohen *et al.* (1982).

Stem stomata

The stem at 7th internodes from base of main stem (during flowering) was

collected from each treatment and peeling of impression was done as followed for siliquae.

Stomata size

Stomatal size was estimated by measuring exterior guard cell length and breadth of five stomata per sample field using 100x magnification (Cohen *et al.* 1982). Calibration was done with the help of stage and ocular micrometer.

Stomatal index (SI)

The stomatal index was calculated as follows:

$$SI = \frac{\text{Number of stomata}}{(\text{No. of guard cell and epidermal cell})} \times 100$$

Leaf ultra structure study:

Ultra structural measurements were done on fully expanded leaf (6th node from the top) of main stem using Transverse Electron Microscope (TEM) technology as reported by (Robertson and Leech 1995). Slices (2-3 mm thickness) were cut from the base of the leaf and fixed in 2.5 per cent glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 1 hr. at room temperature.

The leaf tissues were washed three times in 0.1 M phosphate buffer, pH 7.2, before post-fixation in 1 per cent (v/v) osmium tetroxide in 0.1 M phosphate buffer for two hours at room temperature. After three more washes in 0.1 M phosphate buffer, the tissue was dehydrated through an acetone series and embedded in Spurr' epoxy resin (TABB Laboratories Equipment Ltd., UK, Spurr, 1969). Ultra-thin sections were cut for TEM using a diamond knife. Double staining in uranyl acetate and lead citrate was done for taking observation in electron microscope.

Biochemical studies:

Sampling

Uppermost fully expanded leaves were detached from five different plants

from each treatment at different stages of growth before 12.00 noon when solar PPFD~500 $\mu\text{mol m}^{-2}\text{s}^{-1}$. The biochemical analysis was carried using three replications for each observation. Samples were quickly immersed in ice bucket for enzymatic study and 4 $^{\circ}\text{C}$ temperature was maintained throughout the estimation.

Following biochemical studies were done:

Chlorophyll content

Leaf chlorophyll was extracted by non-maceration method using Dimethyl Sulphoxide (DMSO) (Hiscox and Israelstam, 1979) and light absorption at 663 nm and 645 nm was read in a spectrophotometer. The amount of chlorophyll content was calculated using absorption coefficients.

The fresh leaf material (0.5 g) in a test tube containing 5 ml of DMSO was kept in an oven at 65 $^{\circ}\text{C}$ for about 4 hours. Chlorophyll was extracted in a test tube and the volume was made 10 ml by using DMSO. The OD of the extract was read at 663 nm and 645 nm using spectrophotometer (Model: GS570155, EC India Ltd). The chlorophyll content was determined by the formula as suggested by Arnon (1949) and expressed as mg g^{-1} leaf fresh weight.

$$\text{Chlorophyll a} = \frac{[12.7(D_{663}) - 2.69 (D_{645})] \times V}{1000 \times W}$$

$$\text{Chlorophyll b} = \frac{[22.9(D_{645}) - 4.68 (D_{663})] \times V}{1000 \times W}$$

$$\text{Total Chlorophyll} = \frac{[22.2(D_{645}) + 8.02 (D_{663})] \times V}{1000 \times W}$$

Where, D= Optical density; V= Final volume of the DMSO; W= Weight of the leaf sample

Leaf soluble protein

Leaf soluble protein was estimated by the method of Lowry *et al.* (1951).

Reagents

- i. **Solution A** : 2% sodium carbonate in 0.1 N sodium hydroxide solution.
- ii. **Solution B** : Prepared just prior to its use by mixing equal volume of a) 1% copper sulphate solution and b) 2% sodium potassium tartarate (Rochelle salt).
- iii. **Alkaline cupric tartarate solution (Lowry's reagent)**: Prepared just prior to use by mixing solution A and solution B in 50:1 ratio.
- iv. **Folin–Ciocalteu reagent (IN)**

Fresh leaf material was cut into small pieces (10 mm). The representative sample (0.5 g) extracted with 5 ml of 0.1 M phosphate buffer pH 7.5 using pestle and mortar at 4 °C and centrifuged for 15 minutes in 10,000 g at 4 °C. The supernatant was decanted and equal amount of 15 per cent of fresh trichloro acetic acid (TCA) was added. This solution was kept overnight at room temperature and centrifuged at 3,000 g for 10 minutes. Supernatant was removed by aspiration and pellets were dissolved in 0.1N NaOH and used for protein analysis.

The reaction mixture for protein estimation comprised of 0.5 ml extract, 0.5 ml water, 5 ml of Lowry's reagent and 0.5ml of 1.0 N Folin reagent. This assay mixture was mixed well by vortexing and kept in dark 30 minutes before measurement were made. The absorbance was read at 660 nm vs. prepared blank. The protein content in the samples was calculated and results were expressed as mg protein g⁻¹ FW. by calibration through standard BSA protein.

Estimation of proline

Proline was estimated according to method of Bates *et al.* (1973).

Reagent

- i. **Acid ninhydrin reagent**: 1.25 g of ninhydrin was dissolved in a mixture of warm 30 ml of glacial acetic acid and 20 ml of 6N phosphoric acid. The reagent was stored at 4 °C and used within 24 hours.

- ii. **3% aqueous sulphosalicylic acid**
- iii. **Standard proline solution (25 µg/ml)** : Stock solution was prepared by dissolving 25 mg of proline in 100 ml of distilled water. Working standards were prepared from the stock solution by diluting it.
- iv. **6N phosphoric acid (H_3PO_4)**

Preparation of tissue extract

The leaf samples weighing 0.5 g was ground in 5 ml of 3 per cent sulphosalicylic acid, filtered through 2 layers of muslin cloth and centrifuged at 10,000 g for 10 min. The supernatant was taken and made up to 10 ml with distilled water.

Assay

Sample solution (0.5 ml) was taken in a test tube and made up the volume of 1 ml with distilled water. Five ml of each of glacial acetic acid and ninhydrin reagent were added to it and mixed thoroughly. These tubes were kept in the boiling water bath for 1 hour. The reaction was terminated by placing the tubes on ice bath. The colour produced during the reaction was extracted with 4 ml of toluene by vigorously mixing for 15-20 seconds. The two fractions were separated in a separating funnel. The absorbance of toluene fraction was read at 520 nm in a spectrophotometer using toluene as blank. The reading obtained were then plotted on a graph paper by taking proline concentration on x-axis and absorbance on y-axis.

Free amino acid (FAA)

FAA content of leaves was estimated following the method of Lee and Takahashi (1966).

Reagents

- i. 80% alcohol
- ii. 0.5 M sodium citrate buffer (pH5.6)

- iii. 1% ninhydrin in 0.5 M sodium citrate buffer (pH 5.6)
- iv. 55% glycerol

Preparation of tissue extract

Fresh leaf sample (0.5 g) was homogenized in 10 ml of 80 per cent alcohol. The homogenate was filtered through muslin cloth to remove the debris and the aliquot was heated in water bath at 80 °C for 1 hour. It was then cooled and diluted to 25 ml with distilled water, which was used for the estimation of FAA.

Assay

One milliliter of ninhydrin, 0.2 ml of 0.1 M sodium citrate buffer (pH 5.6) and 0.5 ml of distilled water were added to 2 ml of above filtrate. The reaction mixture was heated on boiling water bath for 20 minutes and cooled. To this solution, 1.3 ml of 55 per cent glycerol was added and final volume was made to 10 ml with distilled water. The absorbance of sample mixture was recorded at 570 nm. The free amino acid content was calculated with the help of the standard curve of alanine and values were expressed as mg of alanine equivalent per g fresh weight.

Free fatty acid (FFA)

For isolation of FFA the leaf sample was initially extracted in 4N HCl and followed by chloroform. This was separated and estimated by following the method of Thimmaiah (1999).

Reagent

- i. 4N HCl
- ii. Chloroform
- iii. 1.0 N Triethanolamine
- iv. Copper reagent (18 ml of 1 N tri-ethanol-amine + 2 ml of 1N acetic acid + 20 ml of 6.45% $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$)
- v. 0.1% Sodium di-ethyl-di-thio-carbamate distilled 2-butanol
- vi. Standard palmitic acid: 4 µg/ml

Isolation

Ten milligram of leaf sample was kept in a glass tube containing 1ml 4N HCl and it was heat-sealed properly. Tubes were kept for 5 hours in boiling water bath with vigorous shaking. The free acid content was extracted with 1ml chloroform.

Assay

Five hundred micro liter (μL) of copper reagent was added in 1ml of extract with vigorous shaking for 2 minutes. This mixture was centrifuged at 2000 g for 10 min at 4°C . The upper layer was aspirated and discarded. 150 μL of 0.1 per cent Sodium di-ethyl-di-thio-carbamate solution was added in lower chloroform layer and absorbance of reaction mixture was recoded at 440 nm. The amount of free fatty acid was calculated by using standard curve prepared from palmitic acid.

Carbohydrates:**Sugars****Extraction**

Sample extraction for estimation of sugar was done according to method reported by McCready *et al.* (1950). Fresh samples of leaves (1.0 g), stem (1.5 g) and root (2.0 g) boiled in 20 ml of 80 per cent (v/v) ethanol in a water bath for 5 minutes. The extract was decanted in a beaker. This ethanol extraction was repeated for 3 more times followed by a final boiling with 20 ml of distilled water for equal time and the supernatant decanted. The combined extract was made up to volume with distilled water in a 100 ml volumetric flask.

Clarification of sugar extract

Fifty milliliter of aliquot of the extract was evaporated in a water bath so that the liquid did not dried out completely. Subsequently, 1 ml of lead acetate was added to precipitate the colloidal substance. It was then filtered into a beaker

and 3 ml of disodium hydrogen phosphate was added and made up to required volume. This aliquot was used for determining the reducing sugar by Nelson's arsenomolybdate method (Nelson, 1944).

Reagents

- i. **Arsenomolybdate colour reagent** : Twenty-five grams of ammonium molybdate $[(\text{NH}_4)_6\text{MO}_{24}\cdot 4\text{H}_2\text{O}]$ was dissolved in 450 ml of distilled water and 21 ml of concentrated H_2SO_4 was added to it and mixed. An aqueous solution of sodium arsenate ($\text{Na}_2\text{HAsO}_4\cdot 7\text{H}_2\text{O}$; 3 g/25 ml) was added and mixed with the above solution and subsequently placed in an incubator at 37°C for 48 hrs.
- ii. **Alkaline copper tartarate (Somogyi's) reagent** : Twenty-four grams of anhydrous sodium carbonate and 12 g of Rochelle salt (Sodium Potassium tartarate) were dissolved in 250 ml of distilled water in a beaker. To this solution, 40 ml of 10 per cent solution, of copper sulphate was added with continuous stirring. This was followed by the addition of 16 g of sodium bicarbonate. In another beaker, 18 g of sodium sulphate was dissolved in 500 ml of hot water and boiled to expel air. After cooling, both these solutions were mixed and diluted to make the volume to 1000 ml.
- iii. **Standard stock glucose solution** : Hundred milligram of glucose in 100 ml distilled water. Working standards were prepared by diluting the stock solution with distilled water (100 $\mu\text{g/ml}$)

Determination of reducing sugars

One milliliter of the aliquot was taken in test tube and made to a uniform volume of 2 ml with distilled water. It was then mixed with 1.0 ml of somogyi's copper reagent and heated in boiling water bath for 12 minutes and then cooled in running tap water. One ml of arsenomolybdate reagent was added to it and volume was made to 10 ml. Absorbance was recorded in a spectrophotometer (Model: GS570155, EC India Ltd) at a wavelength of 530 nm. A blank and two

freshly prepared glucose standards were also included with each set of samples. The sugar content was calculated from a standard curve drawn from freshly prepared glucose solutions and expressed in mg g^{-1} DW.

Total sugar

Five milliliter of extract was hydrolyzed by boiling with half volume of 0.5 N HCl in water bath for 30 min and neutralized at slightly acidic side with NaOH (0.5N). The neutralized extract was made to 10 ml. This solution was used for determining the total sugar. An aliquot of this was analyzed for sugar following the same procedure as described for reducing sugar and expressed in mg g^{-1} DW.

Non-reducing sugar

The reducing sugar was subtracted from the total sugar to obtain the non-reducing sugar content.

Total soluble Sugar

Total soluble sugar (TSS) content was computed by adding reducing and non-reducing sugar content (Allen *et al.*, 1988) and expressed in mg g^{-1} DW.

Starch

Starch content was determined by Anthrone method (Mcready *et al.*, 1950). The dry residue left after sugar extraction was powdered and a known amount of it was hydrolyzed by boiling with 10 ml of 1N HCl in a glycerin bath at 112–115 °C for 30 minutes. The residue was repeatedly washed with distilled water until a negative test of starch by iodine was obtained. The extract was collected and the final volume was made upto 100 ml. An aliquot (0.5–1.0 ml) of the above extract was made to uniform volume of 2.5 ml with distilled water. It was then mixed thoroughly with 10 ml of freshly prepared anthrone reagent (100 mg of anthrone in 100 ml of chilled concentrated sulphuric acid) in a cold water bath. The tubes were then kept in a boiling water bath for 15 minutes and cooled in running tap water. Absorbance was measured at 620 nm in a spectrophotometers

(Model: GS570155, EC India Ltd). A blank and two freshly prepared glucose standards were also included with each set of samples. Starch content was calculated by multiplying the glucose values by 0.9 (Pucher *et al.*, 1948) and expressed in mg g^{-1} DW.

Total non-structural carbohydrate

The total non-structural carbohydrate was computed by adding TSS and starch content (Allen *et al.*, 1988) and expressed in mg g^{-1} DW.

Estimation of carbon

Carbon content was estimated by wet digestion following the modified Walkley-Black method (Walkley and Black, 1934). The dried plant samples were oxidized with a mixture of potassium dichromate and concentrated sulphuric acid using heat of dilution of the acid. The unused potassium dichromate was estimated by back titration with ferrous ammonium sulphate.

Reagents

- i. **Standard potassium dichromate solution (1N):** 49.04 g of AR grade potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) (dried at 105°C for 2 hrs) was dissolved in distilled water and diluted to 1000 ml in a volumetric flask.
- ii. **Ferrous ammonium sulphate solution (0.5 N):** 196.1 g of AR grade ferrous ammonium sulphate [$\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$] was dissolved in about 800 ml of distilled water. 20 ml of concentrated sulphuric acid (H_2SO_4) was added to it and then allowed to cool. The solution was diluted to 1000 ml in a volumetric flask.
- iii. **Diphenylamine indicator:** 0.5 g of diphenylamine [$(\text{C}_6\text{H}_5)_2\text{NH}$] was dissolved in a mixture of 20 ml distilled water and 100 ml concentrated H_2SO_4 (specific gravity 1.84 and concentration $> 96\%$) and was stored in amber colored bottle.
- iv. **Orthophosphoric acid (85% v/v):** Orthophosphoric acid (H_3PO_3) reagent was prepared by diluting it in distilled water.

sample was transferred to different digestion tubes. To this tube, 10 ml of concentrated sulfuric acid was added. One Kjelatab (containing 3.5 g K_2SO_4 + 12.48 mg $CuSO_4 \cdot 5H_2O$) was added to the digestion tube. An assembly of 20 digestion tubes were put in digestion block and covered with fume hood. The digestion temperature was raised to $400^\circ C$ till the samples turned turquoise (about 1 hr). The digestion tubes were removed and allowed to cool for 5-10 minutes. About 50 ml of distilled water was added and mixed well.

Preparation of reagents (Tecator, 1985)

- i. **NaOH solution 40% NaOH:** 10 kg of sodium hydroxide (NaOH) was dissolved in 15 liter of water.
- ii. **Receiving solution:** Hundred gram of H_3BO_3 was dissolved in 10 liters of distilled water. To this, 100 ml bromocresol green solution (100 mg in 100 ml methanol) and 70 ml methyl red solution (100 mg in 100 ml methanol) was added. Finally 5 ml of 4% NaOH was added and the pH adjusted to 4.5.
- iii. **Standard acid (0.1 M HCl):** HCl was standardized by titration against a solution of sodium carbonate (Na_2CO_3). 400 mg (W_1) of oven dried Na_2CO_3 was transferred to a conical flask. It was dissolved in 40 ml of distilled water and 10 drops of methyl red indicator solution (100 mg per 100 ml ethanol) was added. The solution was titrated to pink colour with HCl and the ml of HCl used (A_1) was noted. The solution was boiled for a few minutes and was cooled rapidly to room temperature under running tap water. Titration was continued until the next pink color change occurred. The ml of HCl used (A_2) was noted.

Calculation

$$\text{Molality of HCl} = \frac{18.868 \times W_1}{(A_1 + A_2)}$$

Procedure

Calibration was done with ammonium iron (II) sulphate (mol. wt. 392.14), which contains 7.145 per cent, N. Distillation of 500 mg iron (II) sulphate and titration with standard HCl (0.1M) was done and display value of (ml-blank) was noted. This value was compared with calculated (ml-blank) value for calibration.

$$\text{ml - blank (calculated)} = \frac{\% \text{ N} \times \text{g sample}}{\text{Molarity of HCl} \times 1.401}$$

Where, %N = 7.145 for ammonium iron (II) sulphate, g sample = 0.5g, Molarity of HCl = 0.1 M

The digested extract was made up 100 ml with distilled water. The tube was then fitted to the splash heat for distillation. The instrument was operated as per Tecator (1985) manual. When the distillation and titration cycle is over, the result is displayed on the front panel.

Percent N is calculated as,

$$\% \text{N} = \frac{(V_{\text{sample}} - V_{\text{blank}}) \times M \times 1.401}{\text{g sample}}$$

V_{sample} = the volume (ml) standard HCl for titration of the sample

V_{Blank} = the volume (ml) standard HCl for titration of the sample

M = molarity of solution,

g sample = dry weight of sample

Antioxidative enzymes:

Superoxide dismutase (SOD; EC 1.15.1.1)

The superoxide dismutase (SOD; EC1.5.1.1) activity was determined spectrophotometrically by measuring the inhibition of blue diformazone formation in the presence of riboflavin /nitro-blue tetrazolium (NBT) and light (Beauchamp and Fridovich, 1971).

Reagent

- i. Methionine solution (200 mM)
- ii. Riboflavin solution (75 μ M)
- iii. Nitro-blue tetrazolium solution (1.125 mM)
- iv. EDTA disodium solution (1.5 mM)
- v. Phosphate buffer (50 mM; pH 7.0)
- vi. Phosphate buffer (50 mM; pH 7.8)

Enzyme extraction

Leaf sample weighing 500 mg was homogenized in ice-cold 50 mM phosphate buffer (pH 7.0) containing 0.5 mM EDTA in a pre-chilled pestle and mortar. The homogenate was centrifuged at 4 °C for 30 minutes at 30,000 g. The supernatant was transferred to another tube and used as enzyme extract.

Assay

The assay solution consisted of 0.2 ml methionine (200 mM), 0.2 ml NBT (2.25 mM), 0.2 ml EDTA (1.5 mM), 40 μ L enzyme extract, 2.19 ml phosphate buffer at pH 7.8, and 0.2 ml Riboflavin (75 μ M). Riboflavin was added as the last component. Tubes were shaken and placed 30 cm below a light source, consisting of two 15 W fluorescent tubes. The reaction was started by switching on the light and was allowed to run for 10 min. Light was switched off after 10 min to stop the reaction. The tubes were covered with black cloth immediately after switching off the light. A non-irradiated reaction mixture containing enzyme extract, which did not develop colour, was used as control. Blanks were lacking enzyme in reaction mixture and developed maximum colour. The absorbance of the reaction mixture was read at 560 nm. The volume of the enzyme extract producing 50 per cent inhibition of the reaction was read from the resultant graph. Activity is expressed as unit' mg^{-1} protein. One unit of SOD activity is defined as the enzyme, which causes 50 per cent inhibition of the initial rate of the reaction in the absence of enzyme. (Beauchamp and Fridovich, 1971).

Catalase (CAT; EC1.11.1.6)

Catalase activity in leaves was measured following the method of Teranishi *et al.* (1974) with minor modification. Reaction mixture without enzyme developed maximum colour with titanium reagent. Catalase activity based colour intensity measured spectrophotometrically at 415 nm. The enzyme activity was assayed by estimating the residual H_2O_2 in the reaction mixture using H_2O_2 standard.

Reagent

- i. 0.1 M potassium phosphate buffer (pH 7.5)
- ii. Titanium reagent
- iii. H_2O_2 solution (2 mM)

Enzyme extraction

Fresh leaf sample of 500 mg was macerated in 0.1 M potassium phosphate buffer (pH 7.5). The homogenate was centrifuged at 12,000 g for 20 minutes at 0 °C. The supernatant was collected and used as enzyme extract.

Assay

The reaction mixture comprised of 100 μL of enzyme extract, 1 ml of 2 mM H_2O_2 and 1.9 ml of 0.1 M potassium phosphate buffer in 10 ml test tube. This reaction mixture was incubated at 30 °C for 5 minutes. The reaction was stopped by adding 4 ml of titanium reagent and resultant mixture was centrifuged at 3,000 g for 10 minutes to get clear supernatant. The absorbance was recorded at 415 nm in spectrophotometer. The residual H_2O_2 content in sample were computed with the help of standard curve. Catalase activity was expressed as $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1} \text{ protein}$.

Peroxidase (POD; EC 1.11.1.7)

The Peroxidase activity was measured spectrophotometrically based on the changes in absorption at 470 nm when guaiacol as a substrate in presence of H_2O_2 using the method of Chance and Maehly, 1955.

Enzyme extraction

Fresh leaf sample of 500 mg was homogenized in 10 ml of ice-cold 0.15 M potassium phosphate buffer (pH 6.5). The homogenate was centrifuged at 15,000 g for 25 minutes at 4 °C. The supernatant used as enzyme extract.

Reagent

- i. 0.15 M potassium phosphate buffer (pH 6.5)
- ii. 1% guaiacol prepared in ethanol
- iii. 1% H₂O₂

Assay

The assay mixture comprised of 2.0 ml of the enzyme extract, 60 µL of 1 per cent guaiacol solution in ethanol and 50 µL of 1 per cent H₂O₂ in distilled water. The changes in absorption were measured spectrophotometrically for 120s at 470 nm. The linear part of the curve during the last 60s ($\Delta OD_{470nm} \text{ min}^{-1}$) was used for calculation of the enzyme activity. Horsh radish (HR) peroxidase was used as standard and the enzyme activity was expressed µmol H₂O₂ reduced min⁻¹ mg⁻¹ protein.

Ascorbate peroxidase (APOD, EC 1.11.1.11)

Ascorbate peroxidase activity was measured by a modified spectrophotometric procedure based on the rate of decrease in absorption of ascorbate at 290 nm during ascorbate oxidation (Dalton *et al.*, 1986).

Reagent

- i. 50 mM KH₂PO₄/K₂HPO₄ (pH 7.0)
- ii. 0.5 mM EDTA
- iii. 250 µM ascorbate
- iv. 1.5 mM H₂O₂

Enzyme extraction

Leaf sample weighing 500 mg was homogenized in ice-cold 50 mM

phosphates buffer (pH 7.0) containing 0.5 mM EDTA in a pre-chilled pestle and mortar. The homogenate was centrifuged at 30,000 g in 4 °C for 30 minutes. The supernatant was transferred to another tube and used as enzyme extract.

Assay

The assay was performed in a 1ml cuvette containing 250 µM ascorbate, 50 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ (pH 7.0) and 0.50 EDTA, 1.5 mM H_2O_2 and 100 µL enzyme extract. The rate of decrease in absorption of ascorbate at 290 nm during ascorbate oxidation recorded spectrophotometrically and enzyme activity was expressed as $\text{n mol min}^{-1}\text{g}^{-1}$ FW.

Glutathione reductase (GR, EC 1.6.4.2)

Glutathione reductase activity was measured by a spectrophotometric procedure based on the rate of increase in absorbance at 412 nm in presence of substrate, oxidized glutathion (GSSG) following the method of Smith *et al.* (1988).

Reagent

- i. 0.1 M potassium phosphate buffer (pH 7.5)
- ii. 0.2 M potassium phosphate buffer (pH 7.5)
- iii. 0.5 mM EDTA
- iv. 1 mM EDTA
- v. 3 M DTNB
- vi. 2 mM NADPH
- vii. 20 mM GSSG (oxidized glutathione)

Enzyme extraction

Leaf sample weighing 500 mg was macerated in 10 ml of 0.1 M potassium phosphate buffer (pH 7.5) containing 0.05 mM EDTA and filtered through cheesecloth. The filtrate was centrifuged for 15 minutes at 15,000 g. Supernatant was collected and used as enzyme extract.

Assay

The assay mixture comprised of 0.5 ml of 3 M DNTB (5,5-dithio bis-2-nitro benzoic acid) prepared in phosphate buffer (pH 7.5), 1.0 ml of 0.2 M potassium phosphate buffer pH 7.5 containing 1 mM EDTA), 0.2 ml of 2mM NADPH and 0.2 ml of enzyme extract and final volume was made to 2.9 ml with distilled water. The increase in absorbance at 412 nm was measured immediately after adding GSSG over a period of 10 minutes with an interval of 1 minute. Glutathione reductase activity was expressed as $\Delta OD_{412 \text{ nm}} \text{ min}^{-1} \text{ mg}^{-1} \text{ protein}$.

Enzyme protein estimation

The enzyme protein content was determined according to method of Lowry *et al.*, 1951), using BSA as standard.

Membrane related phenomenon:

Hydrogen peroxide content

Hydrogen peroxide content (H_2O_2) was estimated spectrophotometrically according to method of Mukherjee and Choudhari (1983) using titanium reagent. The standard H_2O_2 was used for calibration. The data was expressed as $\text{H}_2\text{O}_2 \mu\text{g}^{-1} \text{ g DW}$.

Reagent

- i. **Titanium reagent:** 1.0 gram of titanium dioxide and 10 g K_2SO_4 , mixed and digested with 150 ml of concentrated H_2SO_4 for 2–3 hour on hot plate. The digested mixture was cooled and diluted to 1.5 liter with distilled water.
- ii. **Liquid Ammonium Solution**
- iii. **Acetone solution**
- iv. **Sulphuric acid (1.0 M)**

Leaf sample (0.5 g) was homogenized in 10 ml of cold acetone. The homogenate was filtered through whatman No. 1 filter paper. Four milliliters of

titanium reagent was added in the whole extract followed by 5 ml of concentrated ammonium solution to precipitate hydrogen-peroxide titanium complex. This complex was centrifuged for 5 minutes at 10,000 g. The supernatant was discarded and precipitate was dissolved in 1M sulphuric acid. It was re-centrifuged to remove un-dissolved material and absorbance was recorded at 415 nm against blank. Concentration of H_2O_2 was determined using standard curve plotted with known concentration of H_2O_2 .

Lipid peroxidation assay

The level of lipid peroxidation was measured in terms of Thiobarbuteric acid reactive substances (TBARS), a product of lipid peroxidation by following the method of Heath and Packer (1968).

Reagent

- i. Trichloro acetic acid (0.1%)
- ii. Thiobarbuteric acid (0.5%)

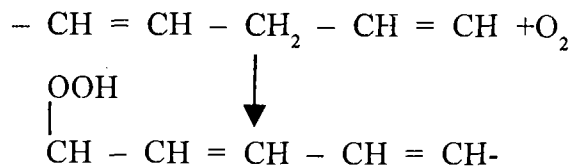
Leaf sample (0.5 g) was homogenized in 10 ml of 0.1 per cent trichloro acetic acid (TCA). The homogenate was centrifuged at 15000 g for 5 minutes. Two ml of aliquot of the supernatant, 4 ml of 0.5 per cent thiobarbuteric acid (TBA) in 20 percent TCA was mixed. The mixture was heated at 95° for 30 minutes and cooled in ice bath. It was centrifuged at 10,000 g for 5 minutes and the absorbance of supernatant was recorded at 532 nm. The value for non-specific absorption at 600 nm was subtracted from value of 532 nm. The MDA content was calculated using its absorption coefficient of $155 \text{ nmol}^{-1} \text{cm}^{-1}$.

$$\begin{aligned} \text{MDA content} &= \frac{\text{OD} \times 6}{0.155} \times \frac{\text{Volume extract}}{2 \times \text{wt. of sample}} \\ &= \text{nmol gm}^{-1} \text{FW} \end{aligned}$$

Lipoxygenase (LOX, Linolate:Oxygen oxidoreductase, EC 1.13.11.12)

The LOX catalyzed reaction can be followed spectrophotometrically by

observing the increase in absorption at 234 nm arising from conjugated double bonds formed during reaction.



Reagent

- i. **Phosphate buffer (0.1 M, pH 6.5)**
- ii. **Substrate solution:** For LOX activity, the substrate was prepared by adding 35 μL of linolenic acid to 5ml of double distilled water containing 50 μL Tween-20. This mixture added with 0.2 M NaOH to dissolved the linolenic acid. When the whole linolenic acid was dissolved, the pH was brought to 6.5 by adding 0.2 M HCl and the final volume was made 100 ml using 0.1 M phosphate buffer (pH 6.5)

Enzyme extraction

Five hundred milligrams of leaf sample was homogenized in 10 ml ice cooled water in a pre-chilled pestle and mortar. The homogenate was allowed to stand for 10 minutes at 4°C and then centrifuged at 15,000 g for 30 minutes at 4 °C. Supernatant was passed through two layers of muslin cloth for removing fatty layer. The resultant clear supernatant was used as crude enzyme extract for assay of LOX.

Assay

The assay mixture comprised of 2.975 ml substrate solution, 25 μL of enzyme extract. The reaction mixture was stirred rapidly and increase in absorbance was measured at 234 nm for 20 minutes in 30-second interval at room temperature (Hildebrand and Hymowitz, 1981). The reaction rate was determined from the slope of the straight-line portion of the curve. Then enzyme activity was expressed as $\Delta A_{234}/\text{min}/\text{mg}$ protein.

Membrane stability index (MSI)

Fresh leaf sample were cut into small discs of uniform size. Representative samples (0.2 g) were weighed and taken in test tubes containing 10 ml of double distilled water. These tubes were incubated at 40 °C in a water bath for 30 minutes and electrical conductivity of the samples were measured (C_1) using Conductivity Bridge. The samples were transferred to the other test tubes and incubated at 100 °C in the boiling water bath for 15 minutes and their electrical conductivity measured as above (C_2). Membrane stability index was calculated and was expressed in percentage using the formula of Premachandra (1990). *

$$MSI = [1 - (C_1 / C_2)] \times 100$$

Relative stress injury (RSI)

Fresh leaf sample of 0.5 g was washed with distilled water and then placed in test tube containing 12 ml of deionized water, kept at 27 °C, and then its EC_1 was recorded. The same was autoclaved at 10 Pi for 10 min then cooled to room temperature and EC_2 was recorded and RSI calculated as formula suggested by Goyal *et al.* (2001) and it was expressed as percentage.

$$RSI = [EC_1 / (EC_1 + EC_2)] \times 100$$

Senescence index (SI)

The number of senescence leaves was counted from vegetative stage (45 DAS) to post flowering (150 DAS). Senescence index at different stages of growth was calculated from data on physiologically active and senesced leaves following the method used by Das and Rabha (2002).

$$SI = \frac{\text{No. of senescence leaves}}{\text{No. of physiologically active leaves} + \text{No. of senesced leaves}} \times 100$$

Statistical analysis:

Statistical analysis of data was done following the method of analysis variance (ANOVA) given by Panse and Sukhatme (1967). The critical difference (CD) values were calculated at 5 per cent probability level.

4. RESULTS

Results describe the interactive effects of elevated CO₂ and moisture stress on the growth, productivity, physiological and biochemical processes of two *Brassica* cultivars i.e. *Brassica campestris* cv. 'Pusa Gold' and *Brassica juncea* cv. 'RH-30' using Free Air CO₂ Enrichment (FACE) technology for CO₂ enrichment. The responses of plants to elevated CO₂ were similar in FACE and OTC grown conditions. Observations recorded from FACE only described in this presentation because FACE is the latest advanced technology of CO₂ enrichment, established at IARI, New Delhi with several advantages over OTC.

Observations are described in the following sequence:

- a. **Response of cultivars to elevated CO₂**
- b. **Variation between the cultivars**
- c. **Response to the moisture stress**
- d. **Response of cultivars to interaction of elevated CO₂ and moisture stress.**

Plant growth:

Height

Elevated CO₂ brought about significant increase in the height of plants in both the cultivars throughout the growth period (Table 5). It ranged from 9.3% (post flowering stage) to 13.5% (flowering stage). The height of plants was greater in 'RH-30' irrespective of treatments. The moisture stress significantly reduced it throughout the growth period. The reduction was maximum at flowering (52%) and flower bud initiation (50%) stage. The stress-induced reduction under ambient and elevated CO₂ condition in 'Pusa Gold' ranged from 31% (vegetative stage)

Table 5. Interactive effect of elevated CO₂ and moisture stress on plant height (cm) at different stages of growth

Treatments	Vegetative			Flower bud initiation			Flowering			Post flowering		
	Pusa Gold	RH-30	Pusa Gold	Pusa Gold	RH-30	RH-30	Pusa Gold	RH-30	RH-30	Pusa Gold	RH-30	RH-30
FACE irrigated	29.60	37.40	64.95	111.90	185.20	100.30	112.90	197.40				
FACE moisture stress	21.70	26.80	48.00	82.23	132.30	72.44	86.40	148.40				
Ambient irrigated	28.10	37.00	63.05	106.75	172.27	98.00	110.90	189.90				
Ambient moisture stress	19.10	25.60	36.35	64.66	106.40	55.20	76.10	127.50				
C.D. at 5% CV.	5.78		12.2		24.77		14.15					
CO ₂	2.1		4.33		10.44		6.44					
CV.x CO ₂	NS		7.44		14.13		NS					
MS	2.39		7.65		6.24		8.76					
CV. x MS	5.12		10.33		11.21		13.36					
CO ₂ x MS	6.44		13.89		15.44		16.24					
CV. x CO ₂ x MS	7.89		16.77		20.67		19.77					

to 44% (flowering) and from 23% (post flowering) to 28% (flowering), respectively. Similarly, in case of 'RH-30' the reduction under ambient and elevated CO₂ conditions ranged from 30% (vegetative) to 38% (flower bud initiation) and 24% (post flowering) to 29% (flowering), respectively.

Stem girth

Significant increase in stem girth was observed due to elevated level of CO₂ (Table 6). The enhancement ranged from 21% (vegetative stage) to 25% (flowering). Stem girth remained significantly higher in 'RH-30' compared to 'Pusa Gold' cultivar. Moisture stress resulted in more than 30% reduction through out the growth period. Stress induced reduction in 'Pusa Gold' under ambient and elevated CO₂ condition varied from 29% (vegetative) to 37% (post flowering) and 15% (post flowering) to 19% (vegetative), respectively, whereas, in 'RH-30' the reduction under ambient and elevated CO₂ ranged between 25% (vegetative) to 35% (post flowering) 14% (flower bud initiation) and 16% (vegetative), respectively.

Number of branch

CO₂ enrichment significantly increased the production of branches in *Brassica* cultivars as 18% primary, 37% secondary, 41% tertiary, and 32% total branch increased (Table 7). The production of branches was significantly higher in 'RH-30' compared to 'Pusa Gold' cultivar. Moisture stress treatment significantly reduced the number of branches in sequence of 29% in primary, 43% in secondary, 47% in tertiary, and 30% in total number of branches. The stress-induced reduction in 'Pusa Gold' under ambient and elevated CO₂ condition was 25% (primary), 31% (secondary), 80% (tertiary) and 34% (total) and 19% (primary), 21% (secondary), 33% (tertiary) and 24% (total number), respectively, whereas, in 'RH-30', it was 22% (primary), 28% (secondary), 33% (tertiary) and 31% (total) under ambient and it was 18% (primary), 19% (secondary), 23% (tertiary) and 22% (total) number of branch per plant in elevated CO₂ condition.

Table 6. Interactive effect of elevated CO₂ and moisture stress on stem girth (cm) at different stages of growth

Treatments	Vegetative		Flower bud initiation		Flowering		Post flowering	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	4.80	6.70	6.67	8.36	7.80	9.20	7.40	8.90
FACE moisture stress	3.90	5.60	5.60	7.03	6.60	7.90	6.30	7.60
Ambient irrigated	4.10	5.90	6.00	7.26	7.10	8.10	6.80	7.80
Ambient moisture stress	2.90	4.40	3.93	4.96	4.60	5.40	4.30	5.10
C.D. at 5% CV.	0.620		0.579		0.234		0.223	
CO ₂	0.316		0.328		0.177		0.201	
CV. x CO ₂	0.447		0.819		0.357		0.284	
MS	0.131		0.235		0.267		0.116	
CV. x MS	0.226		0.353		0.349		0.212	
CO ₂ x MS	0.324		0.473		0.543		0.306	
CV. x CO ₂ x MS	0.461		0.670		0.761		0.432	

Table 7. Interactive effect of elevated CO₂ and moisture stress on different number of branch at different stages of growth

Treatments	Primary			Secondary			Tertiary			Total
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	
FACE irrigated	9.00	9.00	20.00	23.00	3.00	16.00	32.13	48.00		
FACE moisture stress	7.30	7.400	15.60	18.60	2.00	12.30	24.30	37.40		
Ambient irrigated	8.00	7.60	16.00	18.80	2.00	12.90	26.00	37.50		
Ambient moisture stress	6.00	5.90	11.00	13.60	0.00	8.70	17.00	25.80		
C.D. at 5% CV.	1.34		3.12		1.55		5.21			
CO ₂	0.59		1.34		0.45		2.12			
CV. x CO ₂	1.12		1.56		1.21		3.13			
MS	0.54		1.12		0.35		2.78			
CVx MS	0.86		1.87		0.65		4.32			
CO ₂ x MS	1.03		2.34		0.89		5.12			
CV. x CO ₂ xMS	1.67		3.99		1.2		6.98			

Number of leaves

The increase in CO₂ concentration significantly increased the production of leaves ranging from 17% (vegetative) to 28% (flowering) (Table 8). The numbers of leaves were more in 'RH-30' cultivar compared to 'Pusa Gold' throughout the growth period. Moisture stress treatment significantly reduced the number of leaves per plant ranging from 20% (vegetative), to 31% (flowering). Stress induced reduction in 'Pusa Gold' under ambient and elevated conditions ranged from 17% (vegetative) to 29% (post flowering) and from 14% (vegetative) to 21% (flowering), respectively, whereas, in 'RH-30' the range varied from 16% (vegetative) to 29% (flowering) and 14% (vegetative) to 20% (flowering), respectively.

Green leaf area

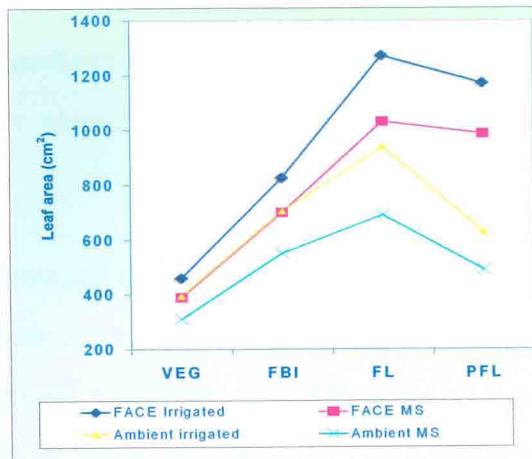
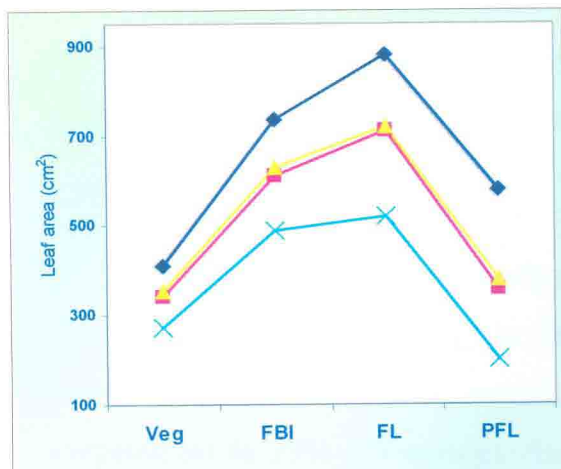
Elevated CO₂ treatment significantly increased the leaf area (25%) throughout the growth period (Fig. 18). The leaf area was greater in the cultivar 'RH-30'. Moisture stress resulted in the reduction of leaf area ranging from 35% (flowering) to 67% (post flowering). The stress-induced reduction in leaf area varied from 23% (vegetative) to 28% (flowering) under ambient condition where as it was ranged from 17% (vegetative and post flowering) to 19% (flower bud initiation) under elevated CO₂ treatment in 'Pusa Gold'. Similarly the reduction in 'RH-30' under ambient and elevated conditions varied from 21% (vegetative) to 26% (flowering) and from 15% (vegetative) to 19% (flowering), respectively.

Leaf area index (LAI)

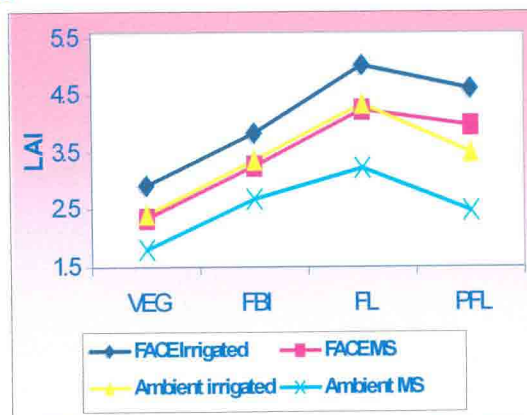
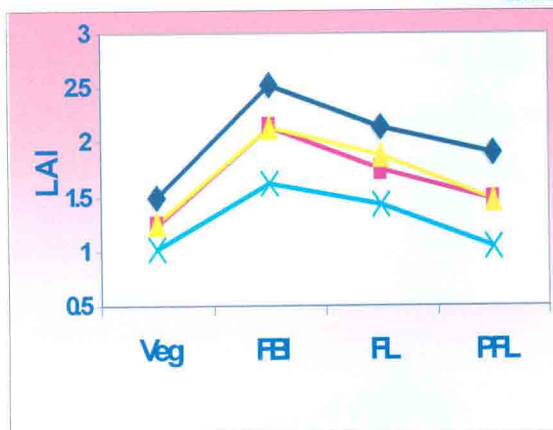
CO₂ enrichment significantly increased the LAI from 17% (flower bud initiation) to 23% (flowering) (Fig. 18). It was markedly higher in case of cultivars 'RH-30' compared to 'Pusa Gold'. Moisture stress treatment significantly decreased the LAI throughout the growth period ranging from 24% (flower bud initiation) to 27% (flowering). Stress induced decrease under ambient and elevated CO₂ conditions ranged from 20% (vegetative) to 40% (flower bud initiation) and 15% (vegetative) to 25% (flower bud initiation), respectively in 'Pusa Gold', whereas,

Table 8. Interactive effect of elevated CO₂ and moisture stress on leaf number at different stages of growth

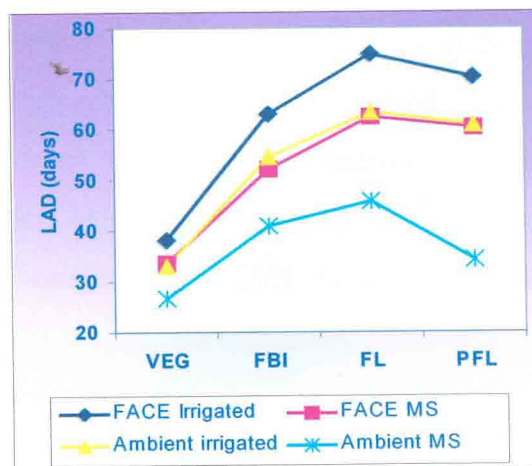
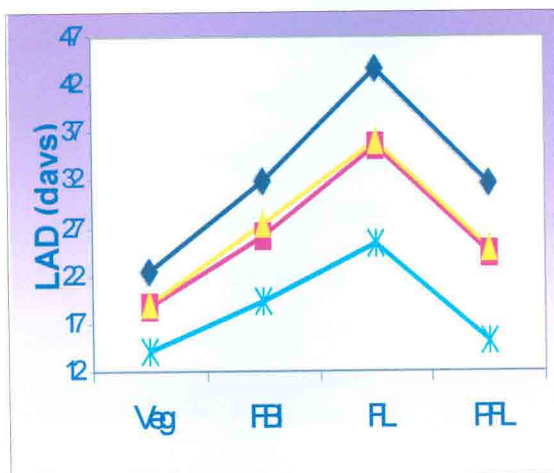
Treatments	Vegetative		Flower bud initiation		Flowering		Post flowering	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	13.50	17.30	18.90	23.30	23.30	29.00	11.70	17.60
FACE drought	11.60	14.90	15.90	19.40	18.50	23.10	9.60	14.60
Ambient irrigated	11.30	15.30	16.00	19.30	18.30	22.60	10.00	14.30
Ambient moisture stress	9.37	12.80	13.30	15.60	14.70	17.20	7.10	10.20
C.D. at 5%								
CV.	1.78		2.17		3.76		2.65	
CO ₂	0.66		1.12		1.54		1.46	
CV. x CO ₂	1.03		1.34		1.89		1.67	
MS	0.64		0.77		1.32		0.97	
CV. x MS	0.89		1.23		2.12		1/05	
CO ₂ x MS	1.12		1.78		2.87		1.88	
CV x CO ₂ x MS	1.89		2.02		3.11		2.12	



leaf area



Leaf area index (LAI)



leaf area duration (LAD)

Pusa Gold

RH-30

Fig. 18. Interactive effect of elevated CO₂ and moisture stress on leaf area, Leaf area index leaf area duration of *Brassica* cultivars

in 'RH-30' it varied from 17% (vegetative) to 28% (post flowering) and 11% (vegetative) to 18% (flowering) under elevated CO₂ condition, respectively.

Leaf area duration (LAD)

CO₂ enrichment significantly increased the leaf area duration from 20% (post flowering) to 27% (flowering) in *Brassica* cultivars (Fig. 18). It was significantly more in 'RH-30'. Moisture stress caused reduction in LAD ranging from 21% (vegetative) to 29% (flowering). Stress induced reduction in LAD under ambient and elevated CO₂ conditions varied from 35% (vegetative) to 43% (flowering) and 19% (vegetative) to 22% (flowering and post flowering), respectively in 'Pusa Gold' whereas, in 'RH-30' the reduction ranged from 23% (vegetative) to 38% (flowering) and 13% (vegetative) to 21% (flower bud initiation), respectively.

Root Characters:

Root length

The length of roots significantly increased (30%) enrichment due to CO₂ (Table 9). It was markedly higher in 'RH-30' cultivar compared to 'Pusa Gold' during crop growth period. Moisture stress treatment significantly reduced the elongation of roots between 30-34% throughout the growth period. The stress induced reduction in root length at ambient and elevated condition varied from 32% (vegetative) to 40% (flowering) and between 14% to 15%, respectively in 'Pusa Gold' whereas, in 'RH-30', it ranged from 29% (vegetative) to 36% (flowering) and 12-14%, respectively.

Root: shoot ratio

The higher-level of CO₂ concentration brought about marked increase in root: shoot ratio ranging from 12% (vegetative) to 37% (flowering) (Table 10). It was greater in 'Pusa Gold' compared to 'RH-30'. Moisture stress caused significant increase in root: shoot ratio varying from 10% (vegetative) to 13% (flowering). The stress induced increase of root: shoot ratio was greater under

Table 9. Interactive effect of elevated CO₂ and moisture stress on root length (cm) at different stages of growth

Treatments	Vegetative		Flower bud initiation		Flowering		Post flowering	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	10.80	14.50	13.50	17.20	16.10	19.85	18.70	22.50
FACE moisture stress	9.21	12.70	11.62	15.03	13.81	17.22	16.09	19.89
Ambient irrigated	9.30	12.42	11.64	14.68	13.86	16.90	16.07	19.12
Ambient moisture stress	6.32	8.80	7.23	9.64	8.22	10.87	10.20	12.90
C.D. at 5%								
CV.	0.43		0.31		0.62		0.73	
CO ₂	0.12		0.29		0.31		0.36	
CV. x CO ₂	0.57		0.43		0.44		0.47	
MS	0.43		0.21		0.53		0.59	
CV. x MS	0.61		0.53		0.67		0.69	
CO ₂ x MS	0.87		0.72		0.75		0.78	
CV. x CO ₂ x MS	1.12		1.01		1.06		1.23	

Table. 10 Interactive effect of elevated CO₂ and moisture stress on root: shoot ratio at different stages of growth

Treatments	Vegetative		Flower bud initiation		Flowering		Post flowering	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	0.364	0.388	0.208	0.154	0.161	0.107	0.165	0.113
FACE moisture stress	0.424	0.434	0.242	0.183	0.191	0.130	0.195	0.138
Ambient irrigated	0.354	0.345	0.185	0.138	0.141	0.098	0.144	0.100
Ambient moisture stress	0.370	0.370	0.199	0.149	0.148	0.102	0.145	0.101
Var.	0.001		0.036		0.021		0.034	
CO ₂	0.015		0.009		0.010		0.01	
CV .x CO ₂	0.081		0.010		0.019		0.13	
MS	0.046		0.030		0.012		0.10	
CV. x MS	0.063		0.040		0.023		0.16	
CO ₂ x MS	0.081		0.050		0.037		0.21	
CV. X CO ₂ x MS	0.116		0.061		0.047		0.30	

elevated CO₂ ranging from was 16% (vegetative) to 19% (flower bud initiation) in 'Pusa Gold' and 12% (vegetative) to 22% (post flowering) in 'RH-30'. There was not significant effect of moisture stress at ambient condition.

Numbers of secondary root

Elevated CO₂ brought about significant increase in the number of secondary root ranging from 28% (post flowering) to 35% (vegetative stage) (Table 11). These were more in 'RH-30' compared to 'Pusa Gold'. Moisture stress treatment significantly reduced the number of secondary roots, ranging from 32% (flowering) to 37% (vegetative). The stress induced reduction under ambient and elevated CO₂ conditions in secondary root production varied between 40% (flower bud initiation) to 46% (vegetative) and 14% (vegetative and flower bud initiation) to 17% (flowering), respectively in 'Pusa Gold' cultivar and from 32% (post flowering) to 39% (flower bud initiation) and 13% (post flowering) to 15% (flower bud initiation), respectively in 'RH-30'.

Volume of roots

The volume of root was significantly increased (36-39%) with increased level of CO₂ throughout the growth period. Root volume of 'RH-30' was markedly higher (Table 12). The moisture stress treatment significantly reduced it ranging from 32% (post-flowering) to 37% (vegetative). The adverse stress effect on root volume under ambient and elevated CO₂ ranged from 38% (vegetative) to 40% (flower bud initiation) and between (18% flower bud initiation to 22% (vegetative stage), respectively in 'Pusa Gold,' whereas, it ranged between 36-37% and 17% (flowering) to 21% (vegetative) in 'RH-30', respectively.

Dry matter distribution:

Leaf dry weight

Elevated CO₂ brought about a significant increase in leaf dry mass ranging from 30% (155 DAS) to 75% (95 DAS) (Fig. 19). The dry weight of leaves was significantly higher in 'RH-30' in comparison to 'Pusa Gold' throughout the growth

Table 11. Interactive effect of elevated CO₂ and moisture stress on secondary root number at different stages of growth

Treatments	Vegetative			Flower bud initiation			Flowering			Post flowering		
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	8.40	13.20	11.20	16.10	13.20	18.10	15.20	20.30				
FACE moisture stress	7.20	11.40	9.40	13.70	11.00	15.60	13.10	17.70				
Ambient irrigated	7.20	11.10	10.10	14.10	11.80	16.00	13.70	17.90				
Ambient moisture stress	3.90	7.10	6.00	8.60	7.10	10.20	8.10	12.00				
C.D. at 5% CV.	0.57		0.65		1.14		0.55					
CO ₂	0.56		0.44		0.83		0.32					
CV. x CO ₂	0.77		0.51		1.18		0.46					
MS	0.49		0.33		0.73		0.44					
CV. x MS	0.68		0.56		0.87		0.54					
CO ₂ x MS	0.78		0.76		1.04		0.63					
CV. x CO ₂ x MS	1.01		0.86		1.47		0.89					

Table 12 Interactive effect of elevated CO₂ and moisture stress on root volume (cc) at different stages of growth

Treatments	Vegetative		Flower bud initiation		Flowering		Post flowering	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	51.30	79.40	78.60	106.30	87.40	112.50	97.30	127.20
FACE moisture stress	40.20	62.30	63.50	86.30	71.20	92.70	79.10	103.90
Ambient irrigated	41.20	63.10	64.27	86.80	69.30	93.20	80.20	104.10
Ambient moisture stress	25.40	40.20	38.60	54.60	41.40	58.40	49.00	65.20
C.D. at 5% CV.	4.85		3.26		7.32		11.84	
CO ₂	3.43		4.42		2.86		6.32	
CV. x CO ₂	4.78		5.54		4.05		8.93	
MS	2.43		4.12		4.72		5.06	
CV. x MS	4.56		5.12		6.68		7.16	
CO ₂ x MS	6.32		7.13		6.68		8.14	
CV. x CO ₂ x MS	9.21		10.04		9.45		10.13	

period. Moisture stress markedly reduced the leaf dry weight ranging from 51% (35 DAS) to 84% (95 DAS). The stress induced reduction in ambient and elevated conditions varied from 40% (35 DAS) to 60% (95 DAS) and from 26% (150 DAS) to 39% (95 DAS), respectively in 'Pusa Gold', and from 30% (150 DAS) to 59% (115 DAS) and from 25% (150 DAS) to 35% (115 DAS) in 'RH-30', respectively.

Stem dry weight

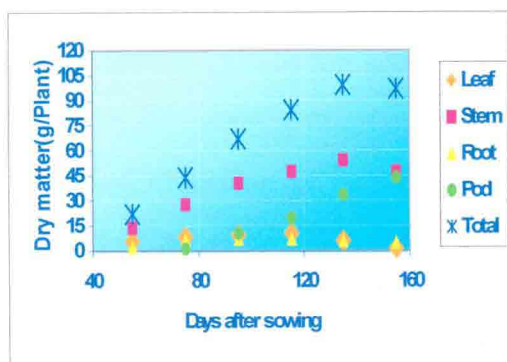
There was significant increase in stem dry weight under elevated CO₂ condition ranging from 18% (55 DAS) to 43% (155 DAS) (Fig. 19). The significantly higher amount of stem dry mass was recorded in 'RH-30'. Moisture stress significantly reduced it ranging from 28% (55 DAS) to 53% (115 DAS). The stress induced reduction in stem dry mass under ambient and elevated CO₂ conditions varied from 30% (55 DAS) to 45% (155 DAS) and from 17% (75 DAS) to 29% (35 DAS), respectively in 'Pusa Gold'. The reduction in 'RH-30' varied from 27% (75 DAS) to 43% (150 DAS) and from 16% (75DAS) to 27% (95 DAS) under ambient and elevated CO₂ condition, respectively.

Root dry weight

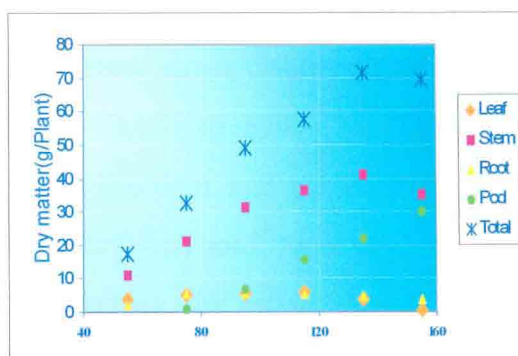
Elevated CO₂ brought about significant increase in dry weight of roots ranging from 23% (155 DAS) to 36% (75DAS, 95DAS and 115DAS) (Fig. 19). Root dry weight was greater in 'RH-30'. Moisture stress significantly reduced the root dry weight ranging from 22% (55 DAS) to 40% (35 DAS and 115 DAS). The stress induced reduction in root dry weight under ambient and elevated condition varied from 28% (55DAS) to 48% (35 DAS) and 16% (115 DAS) to 23% (150 DAS), respectively in 'Pusa Gold'. The reduction in 'RH-30' varied from 24% (75 DAS) to 28% (35 DAS) and from 16% (115 DAS) to 19% (35 DAS), respectively.

Pod dry weight

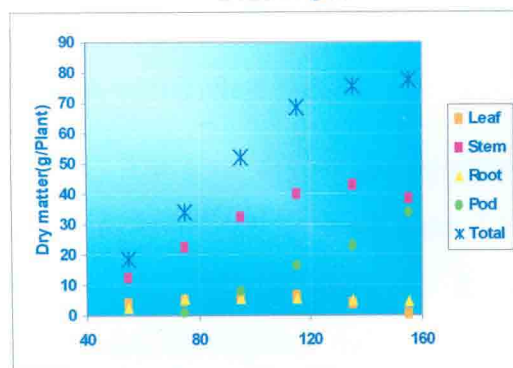
The higher CO₂ concentration brought about marked increase in pod dry weight ranging from 40% (75DAS) to 37% (55DAS) (Fig. 19). It was greater in



FACE irrigated

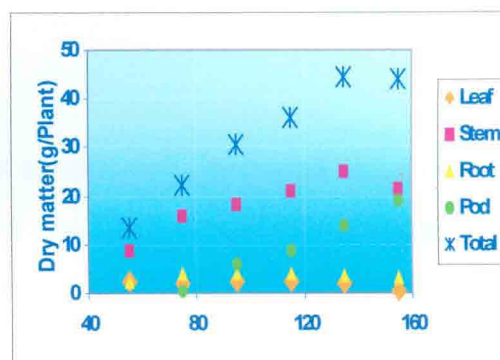


FACE moisture stress

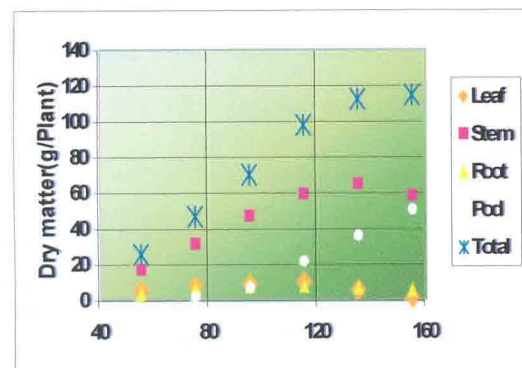


Ambient irrigated

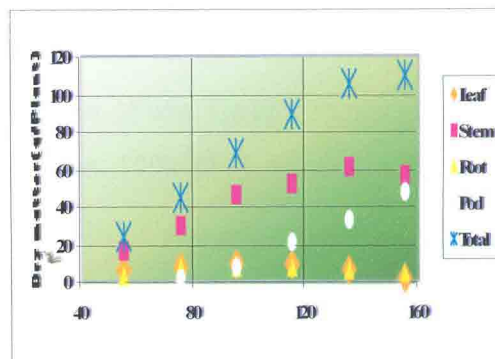
Pusa Gold



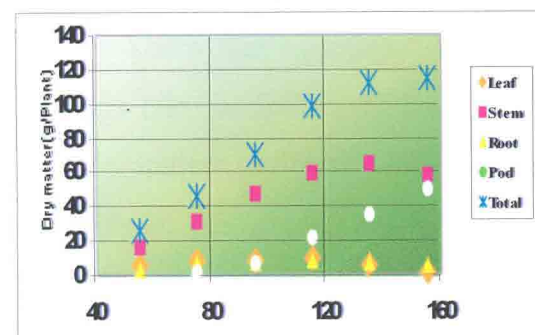
Ambient moisture stress



FACE irrigated

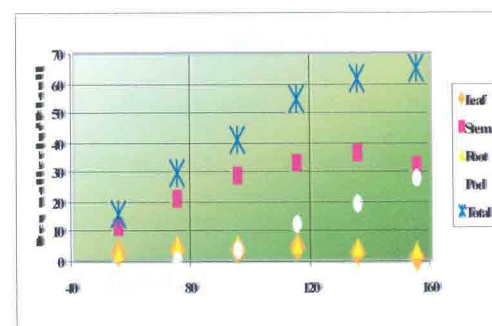


FACE moisture stress



Ambient irrigated

RH-30



Ambient moisture stress

Fig. 19 Interactive effect of elevated CO₂ and moisture stress on dry matter partitioning in *Brassica* cultivars

‘RH-30’ throughout the growth period. Moisture stress caused significant reduction in pod dry weight ranging from 48% (75 DAS) to 57% (55 DAS). The stress induced reduction in pod dry weight under ambient and elevated CO₂ conditions varied from 43% (155 DAS) to 48% (115 DAS) and 18% (115 DAS) to 30% (155 DAS), respectively in ‘Pusa Gold’, whereas, the reduction in ‘RH-30’ varied from 38% (75 DAS) to 42% (95 DAS) and from 17% (115 DAS) to 28% (95 DAS), respectively.

Total plant dry weight

The total dry weight of plant was significantly increased under elevated level of CO₂ condition ranging from 10% (35 DAS) to 44% (95DAS) (Fig. 19). It was higher in ‘RH-30’ cultivar. It was significantly lower under moisture stress condition throughout the growth period ranging from 19% (35DAS) to 56% (115 DAS). The stress induced reduction in total plant dry weight under ambient and elevated condition varied from 18% (35 DAS) to 47% (95 DAS and 115 DAS) and 15% (35 DAS) and 32% (135 DAS) in ‘Pusa Gold’, respectively and from 14% (35 DAS) to 41% (115DAS) and from 12% (115 DAS) to 26% (35 DAS) in ‘RH-30’, respectively.

Growth analysis:

Net assimilation rate (NAR)

There was significant increase in NAR varying from 33% (vegetative stage) to 44% (flower bud initiation) (Table 13). NAR was significantly higher in ‘RH-30’ compared to ‘Pusa Gold’. Moisture stress significantly reduced NAR (30%) throughout the growth period. Stress induced reduction on NAR under ambient and elevated CO₂ condition varied from 34% (post flowering) to 41% (flowering) and 16% (post flowering) to 24% (flowering), respectively in ‘Pusa Gold’, similarly, in ‘RH-30’ the reduction ranged between 33% (post flowering) to 41% (flowering) under ambient condition and between 14% (post flowering) to 21% (flower bud initiation) under elevated CO₂ condition, respectively.

Table 13. Interactive effect of elevated CO₂ and moisture stress on net assimilation rate (g cm⁻² day⁻¹ x10⁻³) at different stages of growth

Treatments	Vegetative			Flower bud initiation			Flowering			Post flowering		
	Pusa Gold			Pusa Gold			Pusa Gold			Pusa Gold		
	RH-30	RH-30	RH-30	RH-30	RH-30	RH-30	RH-30	RH-30	RH-30	RH-30	RH-30	RH-30
FACE irrigated	1.01	1.38	1.39	2.45	2.47	1.76	2.47	1.20	1.92	1.20	1.92	1.92
FACE moisture stress	0.80	1.17	1.07	1.92	1.93	1.33	1.93	1.00	1.64	1.00	1.64	1.64
Ambient irrigated	0.83	1.10	1.12	1.94	1.99	1.40	1.99	0.99	1.69	0.99	1.69	1.69
Ambient moisture stress	0.55	0.70	0.69	1.24	1.16	0.82	1.16	0.65	1.12	0.65	1.12	1.12
C.D. at 5% CV.	0.107		0.118			0.132			0.101			0.101
CO ₂	0.068		0.087			0.101			0.054			0.054
CV. x CO ₂	0.098		0.142			0.156			0.088			0.088
MS	0.078		0.101			0.110			0.057			0.057
CV. x MS	0.101		0.132			0.177			0.086			0.086
CO ₂ x MS	0.137		0.187			0.289			0.117			0.117
CV. x CO ₂ x MS	0.191		0.299			0.376			0.182			0.182

Leaf area ratio (LAR)

Elevated CO₂ reduced the LAR in *Brassica* cultivars ranging from 23% (vegetative stage) to 44% (post flowering) (Table 14). The LAR was lesser in 'RH-30' throughout the growth period. The interactive effect of elevated CO₂ and moisture stress for this character was not significant.

Specific leaf area (SLA)

There was significant reduction ranging from 20% (vegetative) to 27% (Post flowering) in SLA values under elevated CO₂ (Table 15). Specific leaf area was lesser in 'RH-30'. The interaction of CO₂ and moisture stress effect for this character was not significant.

Leaf weight ratio (LWR)

A significant increase in LWR was observed with elevated level of CO₂ throughout the growth period ranging from 28% (vegetative) to 41% (flowering) (Table 16). Higher LWR was recorded at flowering stage onwards in 'RH-30' cultivar. Moisture stress brought about significant reduction in LWR ranging from 25% (vegetative) to 31% (flowering) throughout the growth period. Stress induced reduction on LWR varied from 27% (vegetative and post flowering) to 37% (flowering) under ambient condition where as in elevated CO₂ treatment it ranged from 11% (flower bud initiation and flowering) to 21% (post flowering) in 'Pusa Gold'. Similarly, in 'RH-30' the reduction ranged between 26% (vegetative and post flowering) to 35% (flowering) under ambient condition and between 10% (flower bud initiation) to 16% (post flowering) under elevated CO₂ condition.

Specific leaf weight (SLW)

The higher-level CO₂ brought about marked increase in SLW ranging from 35% (post flowering) to 41% (flowering). Higher SLW was observed in 'RH-30' (Table 17). Moisture stress significantly reduced the SLW ranging from 28% (post flowering) to 41% (flowering). The stress induced reduction in SLW under ambient condition varied from 31% (post flowering) to 48% (flowering) whereas in elevated

Table 14. Interactive effect of elevated CO₂ and moisture stress on leaf area ratio (cm² g⁻¹) at different stages of growth

Treatments	Vegetative		Flower bud initiation		Flowering		Post flowering	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	185.29	149.78	131.88	101.87	85.14	53.96	14.01	10.32
FACE moisture stress	169.49	139.34	123.93	96.06	78.36	52.78	13.24	9.51
Ambient irrigated	212.54	188.14	165.23	133.74	117.92	77.34	20.46	14.42
Ambient moisture stress	208.62	182.69	161.15	127.41	113.67	76.21	19.19	13.71
C.D. at 5%								
CV.	24.98		26.65		27.44		4.67	
CO ₂	15.77		17.99		19.22		2.89	
CV. x CO ₂	20.21		22.34		23.67		3.11	
MS	NS		NS		NS		NS	
CV. x MS	NS		NS		NS		NS	
CO ₂ x MS	NS		NS		NS		NS	
CV. x CO ₂ x MS	NS		NS		NS		NS	

Table 15. Interactive effect of elevated CO₂ and moisture stress on specific leaf area (cm²g⁻¹) at different stages of growth

Treatments	Vegetative			Flower bud initiation			Flowering			Post flowering	
	Pusa Gold	RH-30	Pusa Gold	Pusa Gold	Pusa Gold	Pusa Gold	Pusa Gold	Pusa Gold	Pusa Gold	Pusa Gold	Pusa Gold
FACE irrigated	197.21	155.17	227.14	170.16	181.17	212.18	157.67	304.25	215.12		
FACE moisture stress	205.24	168.29	236.17			220.71	169.73	317.24	238.37		
Ambient irrigated	248.32	204.13	278.92		215.97	263.62	211.72	376.80	287.15		
Ambient moisture stress	265.42	236.21	295.20		231.63	280.31	192.26	400.15	300.26		
C.D. at 5%											
CV.	50.15		55.45				57.65		61.22		
CO ₂	30.34		32.87				35.89		40.72		
CV. x CO ₂	40.66		44.32				46.67		51.33		
MS	NS		NS				NS		NS		
CV. x MS	NS		NS				NS		NS		
CO ₂ x MS	NS		NS				NS		NS		
CV. x CO ₂ x MS	NS		NS				NS		NS		

Table 16. Interactive effect of elevated CO₂ and moisture stress on leaf weight ratio (g g⁻¹ day⁻¹) at different stages of growth

Treatments	Vegetative		Flower bud initiation		Flowering		Post flowering	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	0.166	0.186	0.201	0.219	0.231	0.273	0.093	0.205
FACE moisture stress	0.140	0.163	0.178	0.197	0.204	0.242	0.073	0.177
Ambient irrigated	0.139	0.159	0.173	0.177	0.190	0.226	0.077	0.165
Ambient moisture stress	0.101	0.117	0.118	0.126	0.119	0.146	0.056	0.121
C.D. at 5% CV.	0.005		0.004		0.006		0.005	
CO ₂	0.006		0.005		0.007		0.006	
CV. x CO ₂	0.009		0.009		0.013		0.010	
MS	0.004		0.007		0.009		0.004	
CV. x MS	0.007		0.010		0.012		0.008	
CO ₂ x MS	0.009		0.014		0.017		0.014	
CV. x CO ₂ x MS	0.013		0.019		0.021		0.02	

Table 17. Interactive effect of elevated CO₂ and moisture stress on specific leaf weight (gm⁻² x 10⁻³) at different stages of growth

Treatments	Vegetative		Flower bud initiation		Flowering		Post flowering	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	6.80	8.86	7.69	9.67	11.42	11.95	5.80	9.27
FACE moisture stress	5.68	7.94	6.64	8.40	8.51	9.62	5.01	8.08
Ambient irrigated	5.44	7.74	6.34	8.00	9.01	10.25	4.90	7.85
Ambient moisture stress	3.70	5.54	3.96	5.31	4.71	6.29	3.38	5.42
C.D. at 5%								
CV.	1.04		1.10		1.12		0.78	
CO ₂	0.22		0.49		0.56		0.14	
CV. x CO ₂	0.65		0.78		1.04		0.45	
MS	0.32		0.43		0.65		0.12	
CV. x MS	0.55		0.62		0.88		0.34	
CO ₂ x MS	0.77		0.88		1.12		0.56	
CV.x CO ₂ x MS	0.89		1.01		1.99		0.70	

it ranged from 13% (flower bud initiation and post flowering) and 25% (flowering) in 'Pusa Gold'. The reduction in 'RH-30' ranged between 29% (vegetative) to 38% (post flowering) under ambient condition and from 8% (vegetative) to 13% (flower bud initiation) under elevated CO₂ condition.

Relative growth rate (RGR)

Significant increase in RGR was observed with elevated level of CO₂. The increase ranged from 30% (vegetative stage) to 46% (flower bud initiation) (Table 18). It was higher in 'RH-30'. Moisture stress significantly reduced the RGR ranging from 37% (flower bud initiation) to 36% (flowering). Stress induced reduction for this character varied from 31% (vegetative) to 39% (flowering) under ambient condition and 23% (vegetative) to 28% (flowering) at elevated CO₂ in 'Pusa Gold'. Similarly, in 'RH-30' the reduction ranged between 26% (vegetative) to 36% (flowering) under ambient condition and between 19% (vegetative) to 26% (flowering) under elevated CO₂ condition.

Crop growth rate (CGR)

The higher CO₂ significantly enhanced the CGR in *Brassica* cultivars ranging from 27% (flower bud initiation) to 40% (flowering) (Table 19). It was significantly higher in 'RH-30'. Moisture stress significantly reduced the CGR varying between 34% (post flowering) to 56% (flowering). Stress induced reduction varied from 36% (vegetative) to 41% (flowering) under ambient condition and from 21% (post flowering) to 30% (flowering) under elevated CO₂ in 'Pusa Gold'. Similarly, in 'RH-30' the reduction ranged between 27% (post flowering) to 39% (flowering) under ambient condition and between 19% (post flowering) to 26% (flowering) under elevated CO₂ condition.

Phenological studies:

Crop growth period

Phenological analysis of *Brassica* cultivars indicated significant increase in

Table 18. Interactive effect of elevated CO₂ and moisture stress on relative growth rate (g g⁻¹ day⁻¹) at different stages of growth

Treatments	Vegetative		Flower bud initiation		Flowering		Post flowering	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	0.072	0.093	0.127	0.174	0.144	0.180	0.100	0.123
FACE moisture stress	0.055	0.075	0.094	0.132	0.103	0.133	0.075	0.098
Ambient irrigated	0.059	0.073	0.095	0.123	0.108	0.127	0.077	0.093
Ambient moisture stress	0.041	0.054	0.062	0.085	0.065	0.081	0.052	0.066
C.D. at 5% CV.	0.007		0.013		0.019		0.008	
CO ₂	0.003		0.006		0.008		0.003	
CV. x CO ₂	0.009		0.012		0.019		0.006	
MS	0.004		0.009		0.009		0.004	
CV. x MS	0.006		0.010		0.018		0.007	
CO ₂ x MS	0.008		0.018		0.029		0.010	
CV.x CO ₂ x MS	0.012		0.024		0.035		0.019	

Table 19. Interactive effect of elevated CO₂ and moisture stress on crop growth rate (gm⁻²day⁻¹) at different stages of growth

Treatments	Vegetative			Flower bud initiation			Flowering			Post flowering		
	Pusa Gold	RH-30	Pusa Gold	Pusa Gold	RH-30	Pusa Gold	Pusa Gold	RH-30	Pusa Gold	Pusa Gold	RH-30	RH-30
FACE irrigated	4.35	5.70	16.78	28.06	23.42	30.42	6.90	9.18				
FACE moisture stress	3.26	4.24	12.77	22.21	16.28	22.29	5.44	7.44				
Ambient irrigated	3.73	4.28	13.20	22.26	17.00	21.10	5.30	7.240				
Ambient moisture stress	2.38	2.88	7.99	13.92	10.06	12.89	3.78	5.26				
C.D. at 5% CV.	0.553		4.21		3.21		1.34					
CO ₂	0.487		1.03		1.21		0.98					
CV. x CO ₂	0.689		1.89		2.01		1.23					
MS	0.568		1.01		1.34		0.34					
CV. x MS	0.714		1.87		2.89		0.56					
CO ₂ x MS	0.804		2.01		3.14		0.78					
CV x CO ₂ x MS	1.14		3.21		4.21		1.03					

the duration of vegetative stage (14%) reproductive stage (10%) and whole crop growth period (11%) due to increased CO₂ concentration (Table 20). The crop duration was larger in 'RH-30' compared to 'Pusa Gold'. Moisture stress brought about significant reduction in the phenological duration 15% to vegetative, 10% to reproductive and 11% to whole crop growth period. The stress-induced reduction in 'Pusa Gold' under ambient condition was 22% (vegetative period), 10% (reproductive period) and 13% (crop growth period), whereas in 'RH-30', it was 16% (vegetative period) 15% (reproductive period) and 13% (crop growth period). There was no significant variation for these parameters under elevated CO₂

Seed filling period

The elevated level of CO₂ significantly enhanced (19%) the seed filling period in both early-developed pods (EDP) and late-developed pods (LDP) (Table 20). The seed filling period was longer in 'RH-30' in both EDP and LDP. Moisture stress reduced the seed filling period to 18% in EDP and 20% in LDP. The stress-induced reduction in 'Pusa Gold' 31% in EDP and 31% in LDP, whereas, in 'RH-30' the decrease was 22% (EDP) and 24% (LDP) under ambient. There was no significant effect of elevated CO₂ under moisture stress condition.

Yield attributing characters:

Number of pods per plant

The increased concentration of CO₂ significantly enhanced the production of pods ranging from 27% (75 DAS) to 55% (155 DAS) in *Brassica* cultivars (Table 21). The numbers of pods were significantly greater in 'RH-30'. Moisture stress markedly reduced the production of pods varying between 31% (75 DAS) to 52% (155 DAS). Stress induced reduction in the production of pods ranged between 26% (75 DAS) to 44% (115 DAS) under ambient condition and 19% (75 DAS) to 27% (115 DAS) under elevated CO₂ in 'Pusa Gold'. Similarly, in 'RH-30' the reduction ranged between 24% (75 DAS) to 41% (115 DAS) under ambient condition and between 16% (75 DAS) to 25% (115 DAS) under elevated CO₂ condition.

Table 20 Interactive effect of elevated CO₂ and moisture stress on crop growth period

Treatment	Vegetative (DAS)		Reproductive (DAS)		Seed to maturity (DAS)		Seed filling period (early developed pods, days)	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	43.30	52.60	80.60	93.30	146.00	160.30	61.30	68.30
FACE moisture stress	41.60	50.60	76.30	85.30	133.30	151.60	56.60	63.00
Ambient irrigated	41.30	51.30	76.00	86.30	134.30	151.30	56.50	63.30
Ambient moisture stress	33.60	43.00	66.00	76.30	117.00	132.00	43.00	49.00
CV.	1.555		9.869		10.977		4.922	1.514
CO ₂	2.423		5.091		5.387		2.736	3.292
CV.r. x CO ₂	3.427		7.201		7.635		3.869	4.654
MS	1.954		2.986		4.808		3.939	3.627
CV.. x MS	2.763		4.223		6.799		5.572	5.193
CO ₂ x MS	3.908		5.971		10.158		7.880	7.34
CV..x CO ₂ x MS	5.527		8.445		13.60		11.145	10.79

Table 21 Interactive effect of elevated CO₂ and moisture stress on pod per plant at different period

Treatments	75 DAS			95 DAS			115 DAS			135 DAS		
	Pusa Gold	RH-30	Pusa Gold	Pusa Gold	RH-30	RH-30	Pusa Gold	RH-30	RH-30	Pusa Gold	Pusa Gold	RH-30
FACE irrigated	68.20	144.2	186.45	282.33	308.70	420.40	315.40	467.70				
FACE drought	55.10	120.62	140.00	219.91	227.90	319.20	229.90	352.40				
Ambient irrigated	58.10	118.29	145.10	216.45	232.10	314.70	237.50	340.20				
Ambient drought	42.90	89.14	87.40	134.85	131.90	187.30	133.10	197.40				
C.D. at 5%												
CV.	24.43		34.42		44.45		48.75					
CO ₂	7.44		12.44		27.33		30.11					
CV. x CO ₂	9.56		20.11		44.45		46.23					
MS	3.12		9.78		16.41		16.99					
CV. x MS	3.54		12.99		20.44		22.56					
CO ₂ x MS	4.41		16.23		29.76		28.78					
CV. x CO ₂ x MS	6.23		24.23		38.34		40.33					

Number of seeds per pod

Elevated CO₂ significantly increased (24%) the number of seeds per silique. Cultivar 'Pusa Gold' has more number of seeds per silique irrespective of treatments (Table 22). Moisture stress caused significant reduction (27%) in the production of seeds per pod. The stress-induced reduction in seeds/pod under ambient and elevated CO₂ conditions was 30% and 15%, respectively in 'Pusa Gold' whereas, in 'RH-30' the reduction was 24% and 14%, respectively.

Sink capacity

CO₂ enrichment brought about significant increase (58%) in sink capacity of *Brassica* cultivars. 'RH-30' had higher sink capacity (24%) compared to Pusa Gold (Table 22). Moisture stress significantly reduced (62%) sink capacity of *Brassica* cultivars. The stress-induced reduction under ambient condition was 59% compared to 27% under elevated CO₂ condition in 'Pusa Gold' whereas, in 'RH-30' it was 50% at ambient condition and 24% at elevated CO₂ treatment.

Seed to husk ratio

CO₂ enrichment brought about significant increase (26%) in seed to husk ratio (Table 22). The higher seed to husk ratio was observed in cultivar 'RH-30'. Moisture stress significantly reduced in seed to husk ratio by 38%. The stress-induced reduction under ambient condition was 37% compared to 20% under elevated CO₂ treatment in 'Pusa Gold', whereas, in 'RH-30' the reduction was 36% at ambient condition and 19% at elevated CO₂ treatment.

Harvest Index (HI)

Elevated CO₂ brought about significant increase (14%) in harvest index in *Brassica* cultivars (Table 22). Harvest index was significantly higher in 'RH-30' compared to 'Pusa Gold'. Moisture stress has no significant effect on HI. Interactive effect of moisture and CO₂ was also not significant.

Table 22. Interactive effect of elevated CO₂ and moisture stress on yield attributing characters

Treatments	Seeds / Pod		1000 seeds weight (g)		Seed: husk ratio		Sink capacity		Yield / m ² (g)		HI (%)	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	42.30	20.30	5.11	6.98	1.23	1.89	1322.39	1613.00	728.10	968.30	40.40	44.38
FACE moisture stress	36.00	17.30	4.53	6.20	0.98	1.52	966.91	1219.90	572.40	796.10	39.80	43.93
Ambient irrigated	37.30	17.40	4.570	6.40	1.030	1.68	1042.72	1229.64	624.20	872.30	33.80	40.47
Ambient moisture stress	26.30	13.30	3.27	4.99	0.65	1.07	427.460	619.20	318.10	524.40	34.83	41.12
C.D. at 5%												
CV.	1.18		0.53		0.43		73.64		138.77		3.99	
CO ₂	0.92		0.20		0.08		61.75		35.57		1.67	
CV. x CO ₂	1.30		0.39		0.15		87.33		50.31		NS	
MS	0.83		0.12		0.08		43.51		48.56		NS	
CV. x MS	1.08		0.21		0.11		61.53		68.68		NS	
CO ₂ x MS	1.18		0.34		0.14		70.52		68.68		NS	
CV. x CO ₂ x MS	1.68		0.48		0.20		87.02		97.13		NS	

Yield per meter Square (g m^{-2}):

The increased concentration of CO_2 significantly increased (29%) the seed yield (Table 22). The seed yield in 'RH-30' was higher irrespective of treatment. Moisture stress caused significant reduction (47%) in seed yield. The reduction in seed yield in 'Pusa Gold' was 49% under ambient condition compared to 21% under elevated CO_2 condition. Similarly in the case of 'RH-30' it was 39% under ambient condition and 18% under elevated condition.

Seed weight (1000 seed)

The increased concentration of CO_2 significantly enhanced (21%) the 1000-seed weight in *Brassica* cultivars (Table 22). The 1000-seed weight was 38% more in cultivar 'RH-30' compared to 'Pusa Gold'. Moisture stress caused significant reduction (24%) in 1000-seed weight. The stress-induced reduction on grain weight under ambient condition was 28% where as in elevated CO_2 it was 11% in 'Pusa Gold'. Similarly, in the case of 'RH-30', it was 22% under ambient and 11% under elevated conditions.

Seed quality:

Nitrogen content

The increased concentration of CO_2 significantly reduced (16%) the nitrogen content in *Brassica* seeds (Table 23). Nitrogen content was higher (14%) in 'RH-30' compared to 'Pusa Gold'. The moisture stress induced significant reduction of 18% in the N content of seeds. The reduction under ambient condition was greater in 'Pusa Gold' (30%) than that of 'RH-30' (20%). There was no significant difference in nitrogen content under elevated CO_2 condition.

Carbon content

The increased concentration of CO_2 significantly increased the carbon content (29%) in *Brassica* seeds (Table 23). 'RH-30' had greater carbon content (25%) compared to 'Pusa Gold'. Moisture stress brought about 37% reduction of

Table 23. Interactive effect of elevated CO₂ and moisture stress on seed quality

Treatments	Nitrogen		Carbon		C/N ratio		Protein		Non-reducing sugar		Reducing sugar		Total		Starch		Crude fiber	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	4.31	5.24	31.9	37.4	7.41	7.14	26.9	32.7	22.30	28.3	14.8	19.6	37.1	47.9	78.4	94.3	79.0	89.0
FACE drought	4.24	5.06	22.8	29.5	5.39	5.84	26.5	31.6	16.90	23.4	11.2	15.7	28.1	39.1	59.3	73.4	74.0	83.0
Ambient irrigated	6.17	6.35	25.2	30.2	4.09	4.75	38.2	39.4	17.50	22.7	11.8	15.2	29.3	37.9	63.3	73.8	89.0	103.0
Ambient drought	4.28	5.10	15.9	22.7	3.71	4.46	26.7	31.8	11.30	16.2	7.40	11.3	18.7	27.5	41.9	51.7	86.0	97.0
C.D. at 5% Var.	0.56		3.44		NS		2.12		4.24		2.15		7.74		8.44		7.32	
CO ₂	0.44		1.77		1.11		1.04		2.12		1.09		3.56		5.21		4.44	
Var. x CO ₂	0.62		2.78		1.67		1.97		3.03		1.54		4.78		7.44		8.32	
MS	0.48		1.99		0.67		1.12		1.89		1.84		2.12		3.34		NS	
Var. x MS	0.57		2.45		0.98		1.97		2.12		2.60		3.67		5.77		NS	
CO ₂ x MS	0.83		3.11		1.06		2.01		3.44		2.81		5.44		7.79		NS	
Var. x CO ₂ x MS	1.17		5.01		1.87		2.78		4.12		3.67		8.12		10.44		NS	

it in *Brassica* seeds. The stress-induced reduction in carbon content under ambient condition was 37%, whereas, it was 28% under elevated CO₂ condition in 'Pusa Gold'. Similarly, it was 25% under ambient condition and 21% under elevated CO₂ condition in 'RH-30'.

C/N ratio

Elevated CO₂ significantly increased (51%) the C/N ratio in *Brassica* seeds (Table 23). There was no significant difference between cultivars for this character. Moisture stress treatment markedly reduced C/N ratio (21%). The reduction under ambient condition was greater in 'Pusa Gold' (27%) than that of 'RH-30' (18%). There was no significant difference in C/N ratio under elevated CO₂ condition.

Sugar content

The elevated CO₂ brought about significant increase in the sugar content of seeds (Table 23). The increased was 34% in non-reducing, 33% in reducing and 32% in total sugar. It was more in 'RH-30' compared to 'Pusa Gold'. Moisture stress significantly reduced sugars in seed and reduction was 33% in non-reducing, 35% in reducing and 34% in total sugar content. The stress-induced reduction in sugars under ambient condition was 35% (non-reducing), 37% (reducing) and 36% (total sugar) in 'Pusa Gold', whereas, in elevated CO₂ it was 24% (non-reducing), 24% (reducing) and 24% (total sugar). Similarly, for 'RH-30', under ambient condition it was 28% (non-reducing), 25% (reducing) and 27% (total sugar) compared to 17% (non-reducing), 19% (reducing) and 18% (total sugar) under elevated CO₂.

Starch content

Elevated CO₂ increased the starch content (32%) in *Brassica* seeds (Table 23). Starch content was more in 'RH-30' seed than that of 'Pusa Gold'. Moisture stress brought about 41% reductions. The stress-induced reduction in starch content under ambient and elevated conditions was 33% and 24%, respectively in 'Pusa Gold' and 29% (ambient condition) and 22% (elevated CO₂ condition) in 'RH-30'.

Crude fiber content

The increased concentration of CO₂ significantly reduced (15%) the crude fiber content (Table 23). 'Pusa Gold' seed contain less crude fiber than that of 'RH-30' irrespective of treatment. Crude fiber level in seed was neither affected by the moisture stress nor by its interaction with CO₂.

Oil content:

CO₂ enrichment significantly increased (20%) oil content in *Brassica* seeds (Table 24). Varietal difference for this parameter was not significant. Moisture stress treatment significantly reduced (24%) the oil content in *Brassica* seeds. The stress-induced reduction under ambient and elevated CO₂ condition was 24% and 13%, respectively in 'Pusa Gold' and 27% and 13% at elevated CO₂ condition.

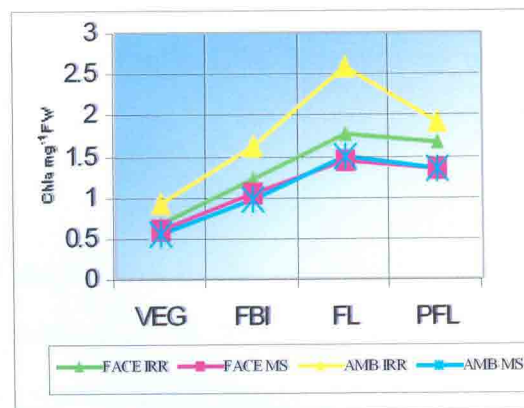
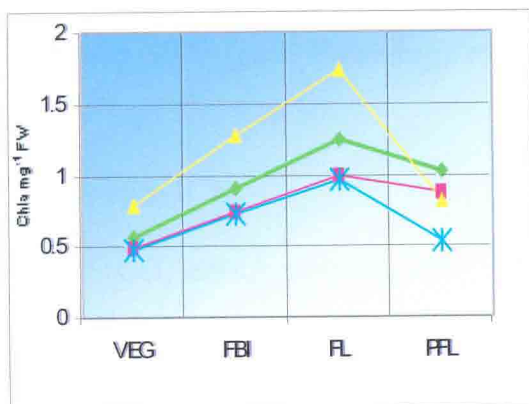
Fatty acid profile:

The fatty acid profile of the *Brassica* seeds was significantly affected by elevated CO₂; it brought about marked reduction in palmitic acid (55%), stearic acid (39%), oleic acid (26%), linolenic acid (20%) and erucic acid (12%), whereas, the linoleic acid (11%) content was increased (Table 24). However, the cultivar difference for stress effect and the interactive effect of CO₂ and moisture stress were not significant for fatty acid profile.

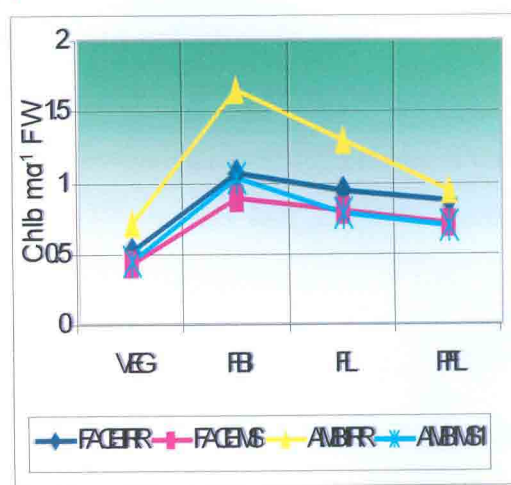
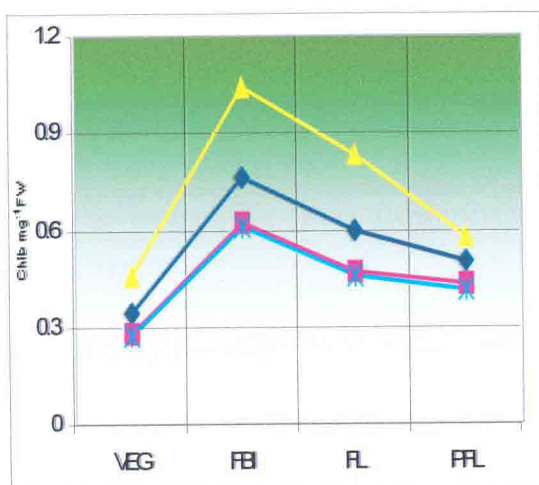
Pigments:

Chlorophyll a

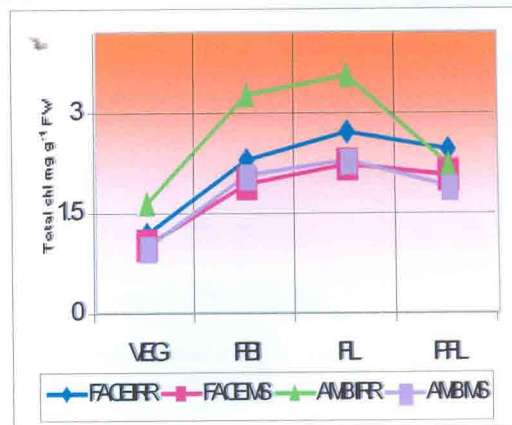
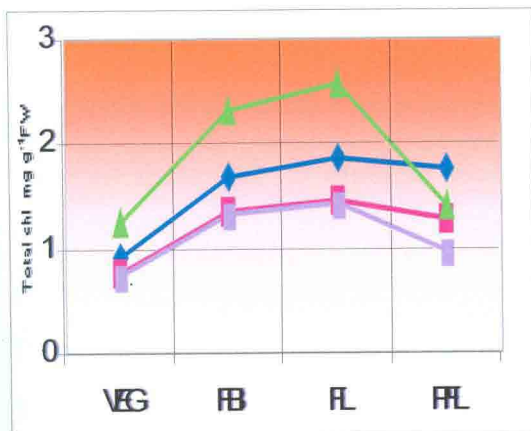
CO₂ enrichment significantly reduced the chlorophyll a content ranging from 17% (post flowering) to 26% (flowering) in the leaves of *Brassica* cultivar (Fig. 20). The chlorophyll a content was more in 'RH-30' cultivar irrespective of treatments. Moisture stress significantly decreased it to 36% at post flowering and to 64% at flower bud initiation. The stress induced reduction in ambient and elevated CO₂ conditions varied between 34% (post flowering) to 44% (flowering) and between 14% (vegetative) to 21% (flowering), respectively in 'Pusa Gold' and



Chlorophyll a



Chlorophyll b



Pusa Gold

Total chlorophyll

RH-30

Fig. 20 Interactive effect of CO₂ and moisture stress on chlorophyll content in leaves of *Brassica* cultivars

between 30% (post flowering) to 42% (flowering), 12% (vegetative) to 19% (at flowering) in 'RH-30'.

Chlorophyll b

CO₂ enrichment caused marked depression in the chlorophyll b content in the leaves ranging from 15% (post flowering) to 25% (flowering) (Fig. 20). Chlorophyll b was more in 'RH-30'. Moisture stress treatment significantly reduced the chlorophyll b content from 35% (post flowering) to 60% (flower bud initiation) during the crop growth period. The stress induced reduction in ambient and elevated CO₂ conditions varied from 30% (post flowering) to 45% (flowering) and between 17% (vegetative and post flowering) to 21% (flowering), respectively in 'Pusa Gold' and between 26% (post flowering) to 44% (flowering) and 15%, respectively in 'RH-30'.

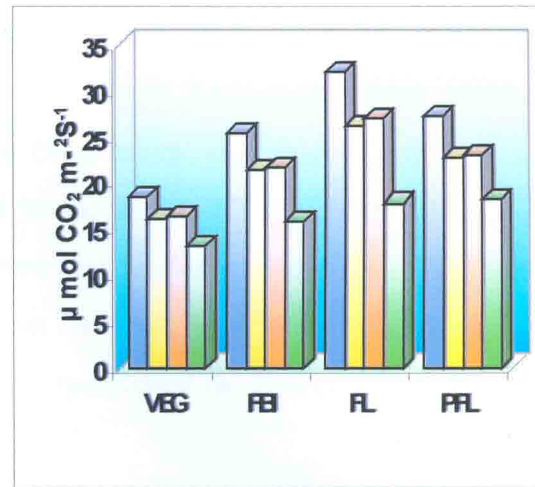
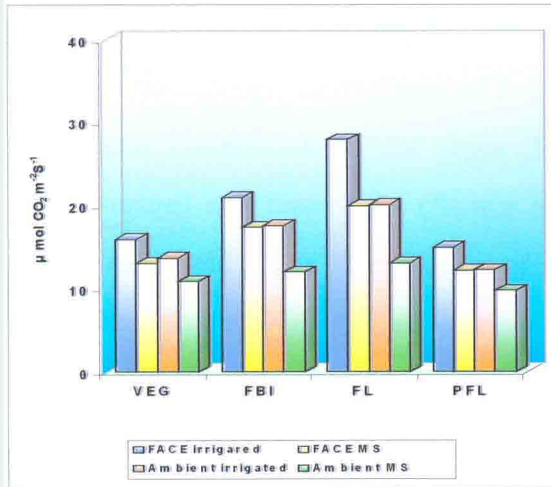
Total chlorophyll

The elevated CO₂ markedly reduced the total chlorophyll content and the reduction ranged from 16% (post flowering) to 34% (flower bud initiation) (Fig. 20). Total Chlorophyll content was more in 'RH-30' irrespective of treatment. Moisture stress significantly decreased the total chlorophyll content ranging from 32% (post flowering) to 44% (flowering) at various stages of growth. The stress induced reduction in ambient and elevated CO₂ conditions varied between 32% (post flowering) to 44% (flowering) and between 15% (vegetative) to 30% (post flowering), respectively in 'Pusa Gold' and between 29% (post flowering) to 40% (vegetative) and 13% (vegetative) to 31% (post flowering), respectively in 'RH-30'.

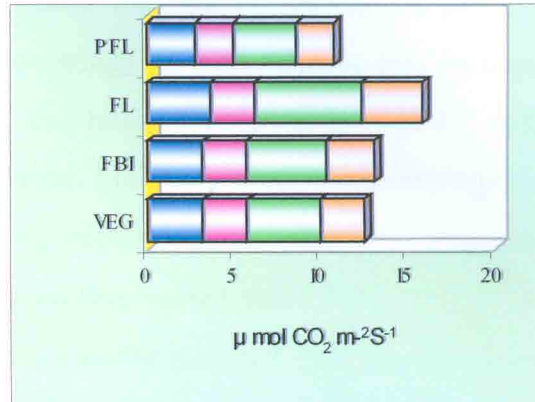
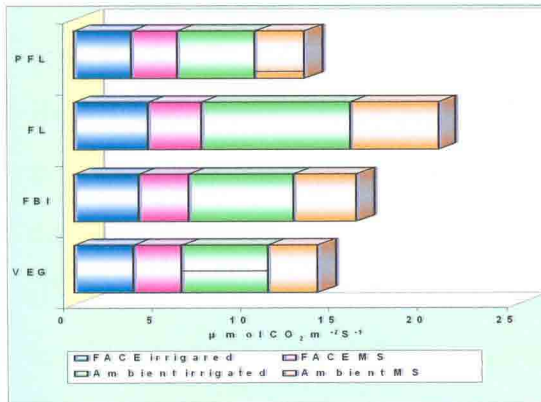
Gas exchange:

Photosynthesis

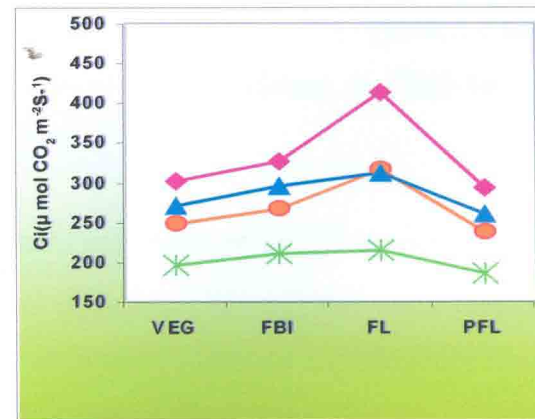
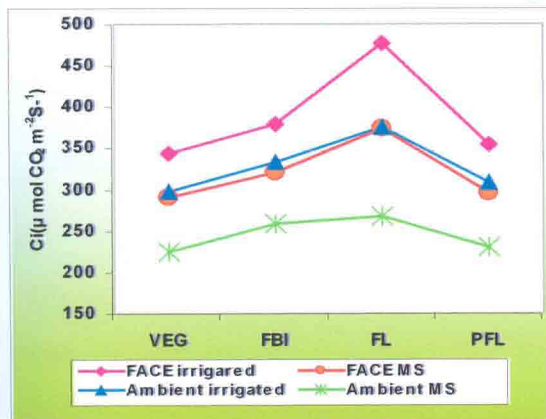
Elevated CO₂ brought about significant increase in the rate of photosynthesis in *Brassica* leaves varying from 19% (vegetative) to 36% (flowering) (Fig. 21). The rate of photosynthesis was more in 'RH-30' irrespective of treatment. Moisture



Photosynthesis



Respiration



Pusa Gold

Internal CO_2 concentration (Ci)

RH-30

Fig 21 Interactive effect of elevated CO_2 and moisture stress on photosynthesis, respiration and internal CO_2 concentration (Ci) in *Brassica* cultivars.

stress significantly decreased the rate of photosynthesis from 20% (vegetative) to 39% (flowering) throughout the growth period. Reduction under ambient and elevated conditions varied from 21% (vegetative and post flowering) to 35% (flowering) and from 17% (flower bud initiation) to 29% (flowering), respectively in 'Pusa Gold'. The stress induced depression under and elevated CO₂ conditions ranged between 20% (vegetative and post flowering) to 34% (flowering) and from 13% (vegetative) to 18% (flowering), respectively in cultivar 'RH-30'.

Respiration

CO₂ enrichment significantly reduced the rate of respiration per unit leaf area from 21% (Vegetative and post flowering) to 40% (flower bud initiation) in *Brassica* cultivars (Fig. 21). The rate of respiration was lower in 'RH-30' compared to 'Pusa Gold' throughout the growth period. Moisture stress treatment significantly decreased the rate of respiration ranging between 42% (post flowering) to 58% (flowering). Reduction under ambient condition varied from 36% (post flowering) to 41% (vegetative and flowering) whereas under elevated CO₂ condition between 17% (vegetative) to 27% (flowering) in 'Pusa Gold'. It ranged between 36% (post flowering) to 34% under ambient condition and between 20% (vegetative and post lowering) to 29% (flowering) under elevated CO₂ condition in 'RH-30'.

Intercellular CO₂ concentration (Ci)

Elevated CO₂ brought about significant increase in Ci ranging from 17% (flower bud initiation) to 37%, (flowering) (Fig. 21). 'RH-30' had more intercellular CO₂ throughout the growth period. Moisture stress treatment significantly decreased it between 28% (vegetative) to 37% (flowering). Stress induced reduction under ambient and elevated conditions varied from 27% (vegetative) to 31% (flowering) and between 18% (post flowering) to 23% (flowering), respectively in 'Pusa Gold'. The reduction between 22% (flower bud initiation) to 28% (flowering) under ambient condition and between 15% (vegetative) to 21% (flowering) under elevated CO₂ condition in 'RH-30' was observed.

Ratio of photosynthesis and respiration (P_N/R_d)

The ratio of P_N/R_s was considerably increased increase between 38% (vegetative), to 78% (flowering) due to CO_2 enrichment (Fig. 22). The P_N/R_s was higher in 'RH-30' throughout the growth period. Moisture stress treatment significantly increased the P_N/R_s ratio varying from 10% (post flowering) to 18% (vegetative). The stress induced increase in P_N/R_s varied from 10% (flowering) to 32% (vegetative) in 'Pusa Gold' whereas, in 'RH-30' it was 13% to 36% (vegetative). The interactive effect of moisture stress and elevated level of CO_2 was not significant.

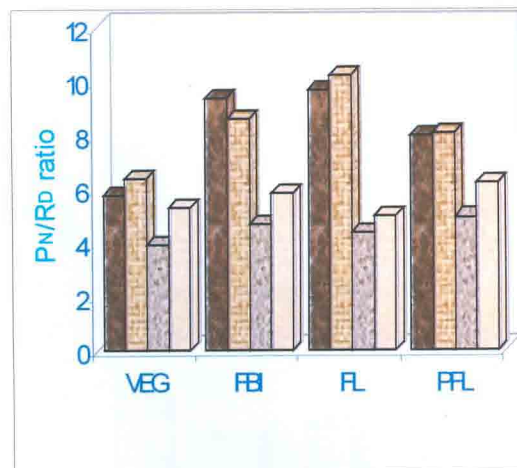
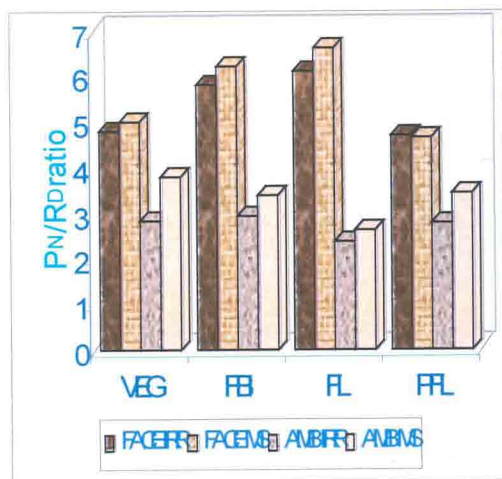
Stomatal conductance (gs)

The stomatal conductance was markedly reduced by elevated level of CO_2 concentration varying from 29% (post flowering) to 48% (flower bud initiation) (Fig. 22). The stomatal conductance of 'Pusa Gold' was more. Moisture stress significantly reduced the stomatal conductance ranging between 15% (vegetative flowering and post flowering) to 21% (flower bud initiation). The interactive effect of moisture stress and CO_2 was not significant.

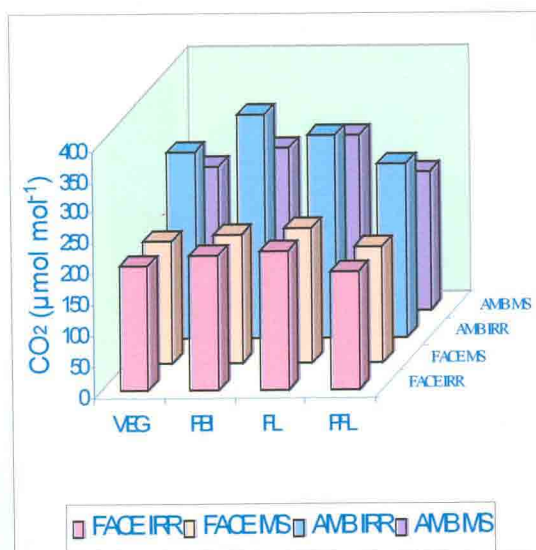
Carbohydrate content in Leaves:

Leaf non-reducing sugar

CO_2 enrichment brought about significant increase in the non-reducing sugar content from 18% (vegetative) to 26% (flowering) (Fig.23). It was significantly greater in 'RH-30'. Moisture stress treatment significantly decreased the non-reducing sugar between 30% (vegetative), 58% (flowering). The reduction under ambient and elevated CO_2 conditions varied from 25% (vegetative) to 38% (flowering) and from 15% (vegetative) to 26% (flowering), respectively in 'Pusa Gold', whereas, between 24% (vegetative) to 35% (flowering) and 14% (vegetative) to 35% (flowering), respectively in 'RH-30'.

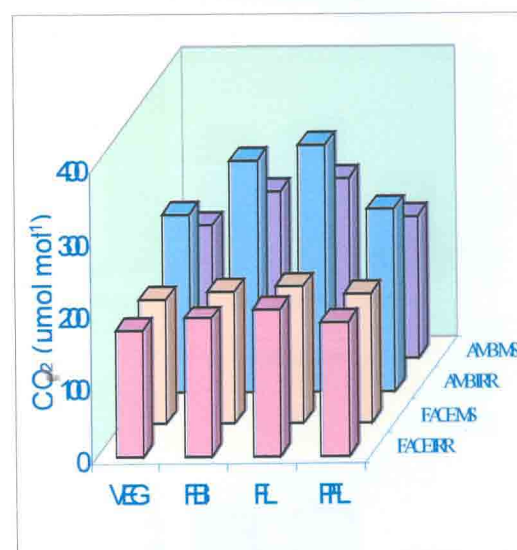


P_n/R_d Ratio



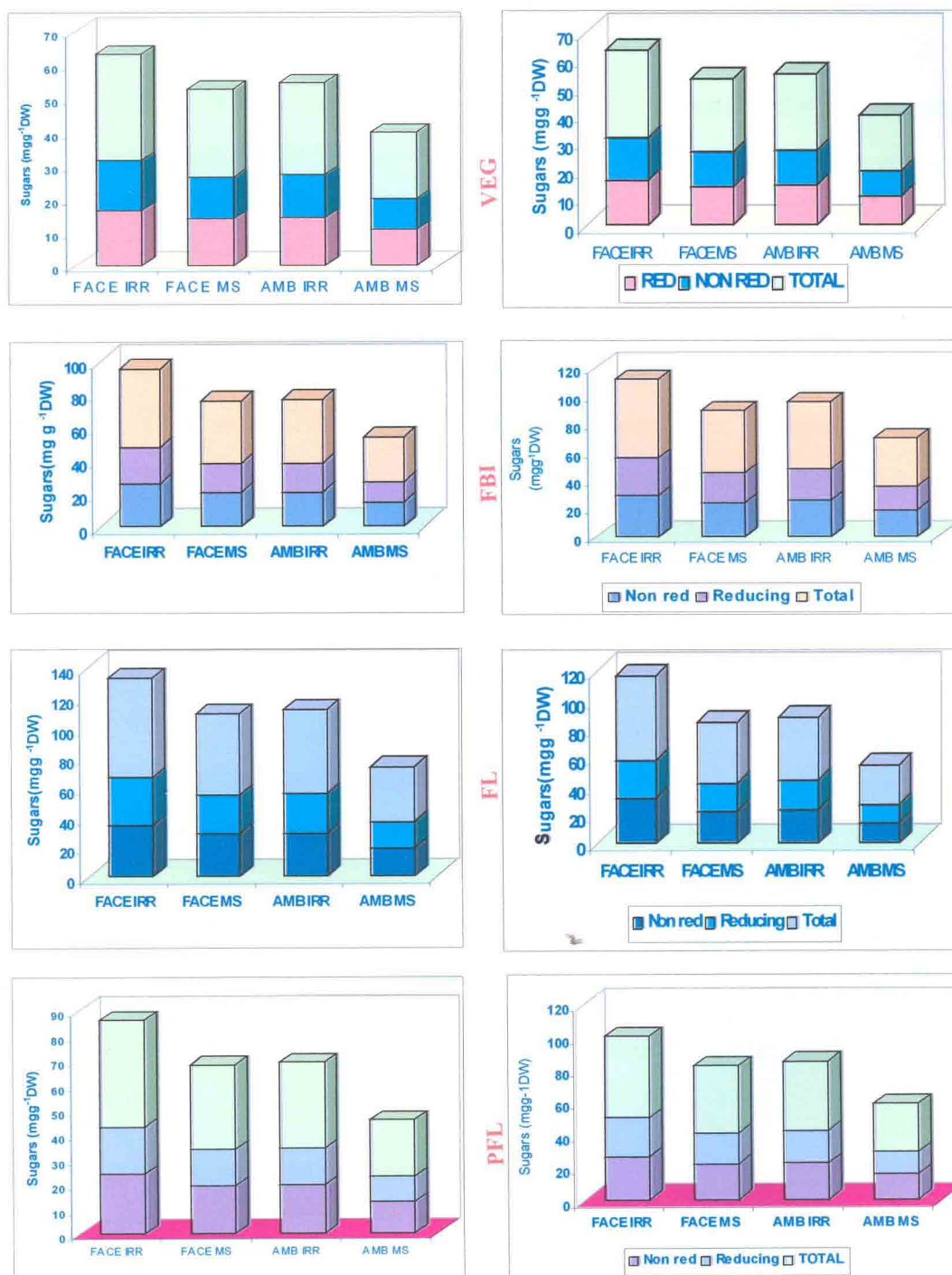
Pusa Gold

Stomatal conductance



RFL-30

Fig. 22 Interactive effect of elevated CO_2 and moisture stress on P_n/R_d Ratio and stomatal conductance of *Brassica* cultivars



Pusa Gold **RH-30**
Fig 23 Interactive effect of elevated CO₂ and moisture stress on distribution of sugars in the leaves of *Brassica* cultivars

Leaf reducing sugar

Significant increase in the reducing sugar content of the leaves ranging from 20% (vegetative) to 26% (flowering and post flowering) was observed due to CO₂ enrichment (Fig. 23). Reducing sugar was significantly more in 'RH-30'. Moisture stress brought about significant decrease in the reducing sugar content varying from 36% (vegetative stage) to 56% (flowering). It varied from 29% (vegetative) to 36% (flowering) and from 17% (vegetative) to 27% (flowering) under ambient and elevated CO₂ condition, respectively in 'Pusa Gold' whereas between 26% (vegetative) to 33% (flowering) 17% (vegetative) to 25% (flowering) under ambient and elevated CO₂ conditions, respectively in 'RH-30'.

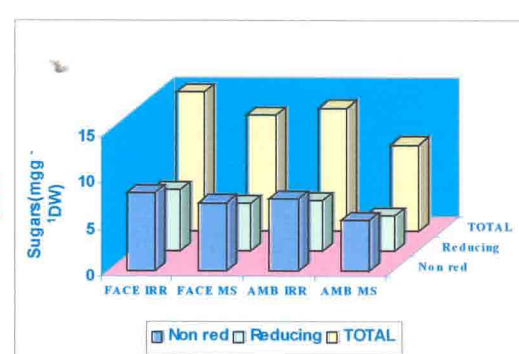
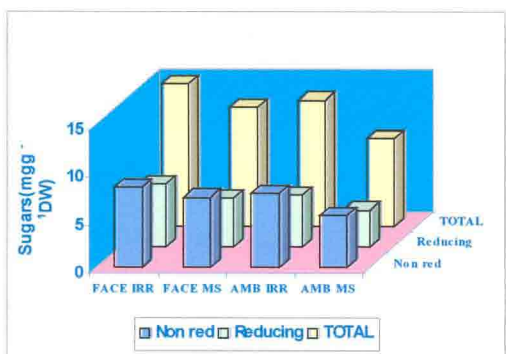
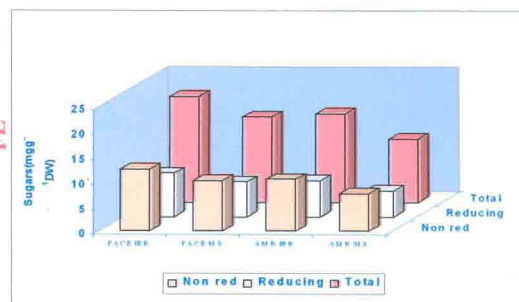
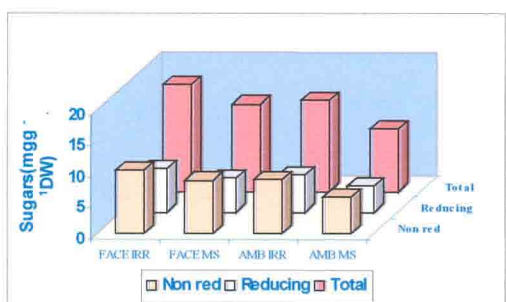
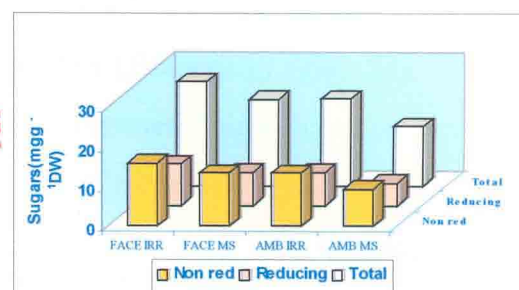
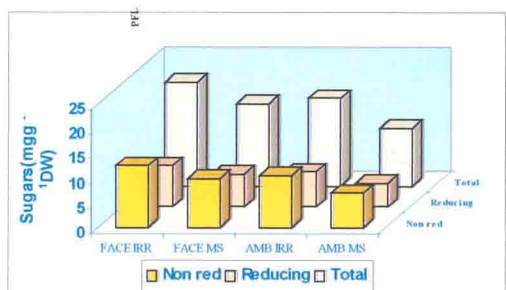
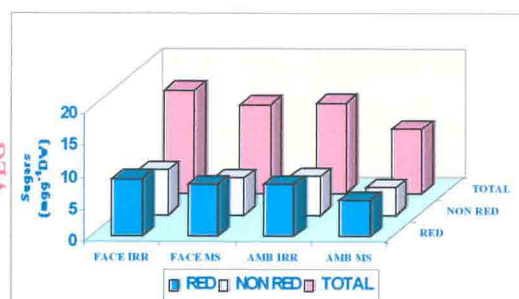
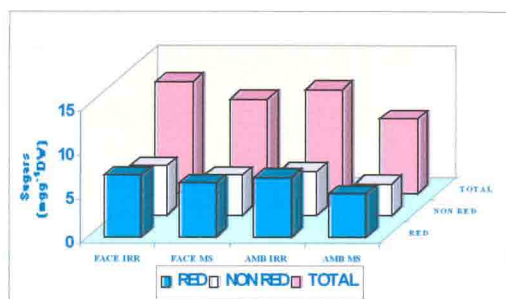
Leaf total sugars

CO₂ enrichment brought about significant increase in the total sugar content varying from 19% (vegetative) to 28% (flowering) throughout the growth period (Fig. 23). It was significantly greater in 'RH-30'. Moisture stress significantly decreased the total sugar content between 33% (vegetative) to 57% (flowering). Reduction under ambient and elevated CO₂ condition varied from 27% (vegetative) to 37% (flowering) and from 16% (vegetative) to 20% (post flowering), respectively in 'Pusa Gold' and in case of 'RH-30' it ranged between 26% (vegetative) to 34% (flowering) and 15% (vegetative) to 18% (flowering) under ambient and elevated CO₂ condition, respectively.

Carbohydrate content in stem:

Stem non-reducing sugar

CO₂ enrichment brought about significant increase in non-reducing sugar content in the stem of *Brassica* cultivars between 16% (vegetative) to 24% (flower bud initiation and flowering) (Fig. 24). Non-reducing sugar content was more in the stem of 'RH-30' cultivar compared to 'Pusa Gold' throughout the growth period. Moisture stress significantly reduced the non-reducing sugar ranging from 28% (vegetative) to 48% (flower bud initiation). This reduction under ambient and



Pusa Gold

RH-30

Fig 24 Interactive effect of elevated CO₂ and moisture stress on sugars distribution in stem of *Brassica* cultivars

elevated conditions varied from 27% (vegetative) to 34% (flower bud initiation) and from 12% (vegetative) to 21% (flower bud initiation), respectively in 'Pusa Gold', whereas, between 27% (vegetative) to 30% (flower bud initiation) and between 10% (vegetative) to 17% (flower bud initiation) in 'RH-30'.

Stem reducing sugar

CO₂ enrichment brought about significant increase in the reducing sugar content ranging from 19% (vegetative) to 25% (flowering) (Fig. 24). It was more in the stem of 'RH-30'. Moisture stress treatment significantly decreased the reducing sugar from 33% (vegetative) to 44% (flower bud initiation). The reduction under ambient and elevated CO₂ conditions varied from 28% (vegetative) to 32% (flower bud initiation) and from 19% (vegetative) to 21% (flowering), respectively in 'Pusa Gold', whereas, between 27% (vegetative) to 31% (flower bud initiation) and 17% (vegetative) to 20% (post flowering), respectively in 'RH-30'.

Stem total sugar

The higher CO₂ level brought about significant increase in the total sugar content of stem ranging from 17% (vegetative), 25% (flower bud initiation). Total sugar content was more in the stem of 'RH-30' (Fig. 24). Moisture stress treatment significantly decreased the total sugar between 30% (vegetative) to 47% (flower bud initiation). This reduction under ambient and elevated CO₂ conditions varied from 28% (vegetative) to 33% (flower bud initiation) and 15% (vegetative) to 20% (flower bud initiation), respectively in 'Pusa Gold', whereas, between 27% (vegetative) to 31% (flower bud initiation) and 13% (vegetative) to 18% (flowering) in 'RH-30', respectively.

Carbohydrate content in roots:

Root non-reducing sugar

CO₂ enrichment significantly increased the non-reducing sugar content in roots from 18%, (vegetative) to 25% (flower bud initiation) throughout the growth period. Non-reducing sugar was more in the roots of 'RH-30' (Fig. 25). Moisture

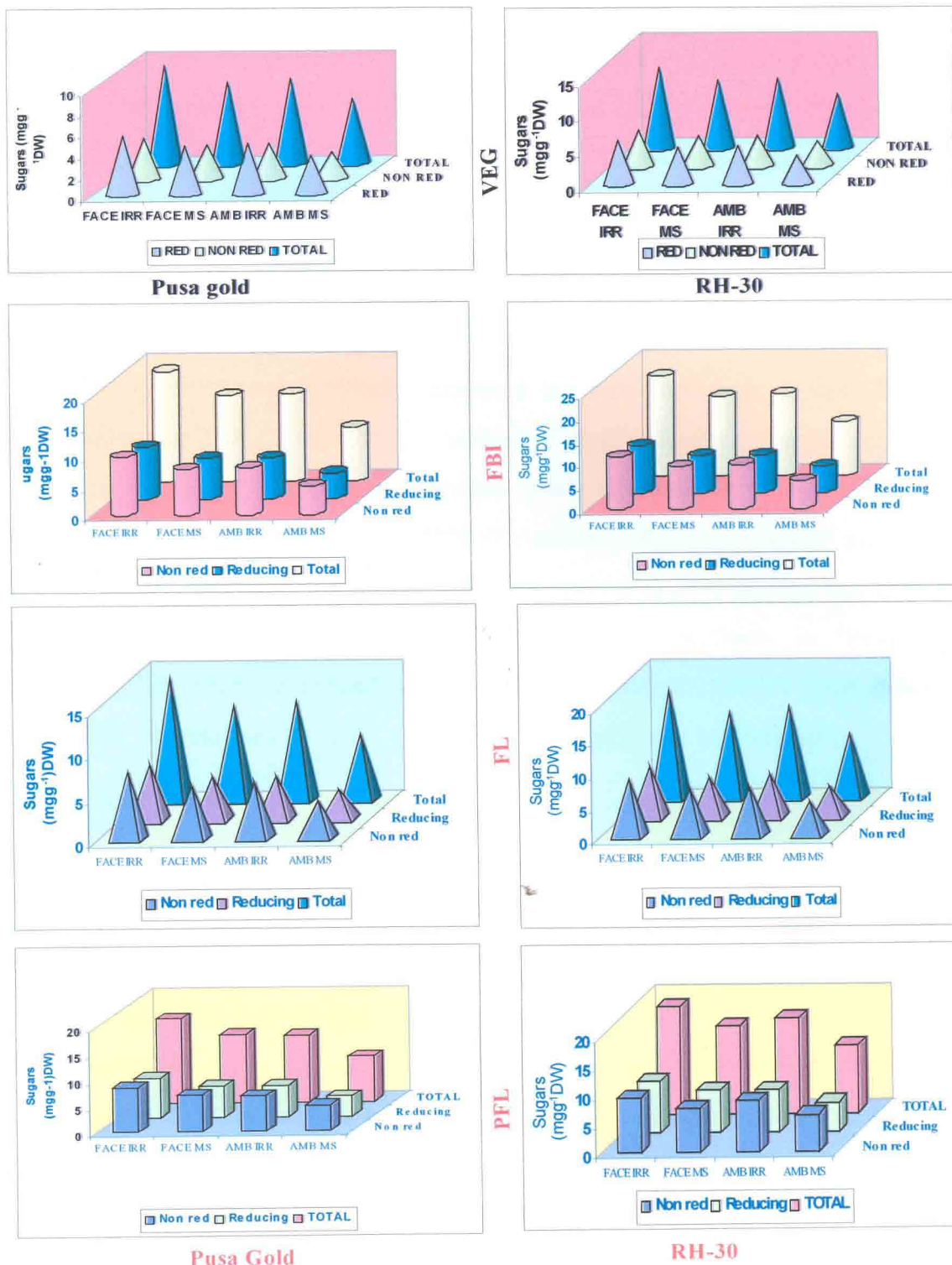


Fig. 25 Interactive effect of elevated CO₂ and moisture stress on sugars distribution in roots of *Brassica* cultivars

stress treatment significantly decreased it ranging from 30% (vegetative) to 57% (flower bud initiation). This reduction under ambient and elevated CO₂ conditions varied from 24% (vegetative) to 38% (flower bud initiation) and 17% (vegetative) to 21% (flower bud initiation), respectively in 'Pusa Gold', whereas, in 'RH-30' the reduction varied between 23% (vegetative) to 36% (flower bud initiation) and 16% (vegetative) to 20% (flower bud initiation), respectively.

Root reducing sugar

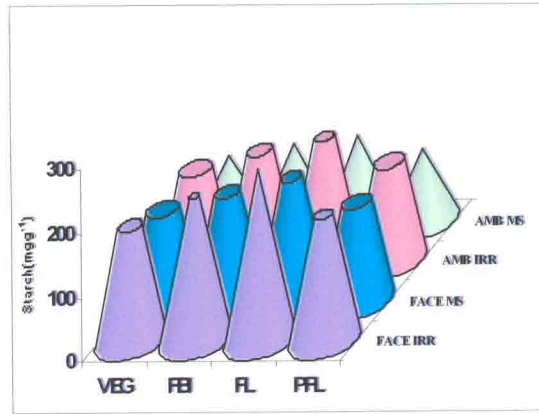
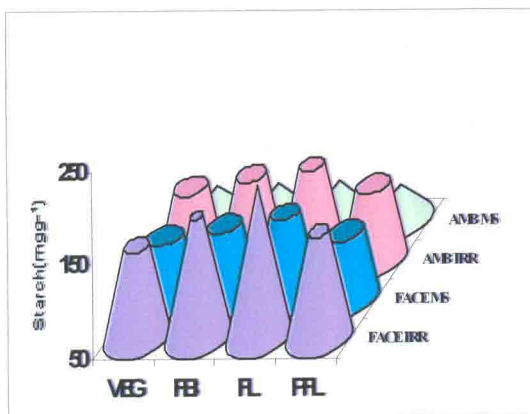
CO₂ enrichment markedly increased the reducing sugar content from 20% (vegetative) to 39% (flower bud initiation) (Fig. 25). Reducing sugar content was more in the roots of 'RH-30'. Moisture stress significantly decreased it, which varied from 33% (Vegetative) to 48% (Flowering). Reduction under ambient and elevated CO₂ condition varied from 23% (vegetative) to 37% (flower bud initiation) and from 17% (vegetative) to 23% (flowering), respectively in 'Pusa Gold', whereas, this reduction ranged between 21% (vegetative) to 30% (post flowering) and 15% (vegetative) to 23% (flowering), respectively in 'RH-30'.

Root total sugar

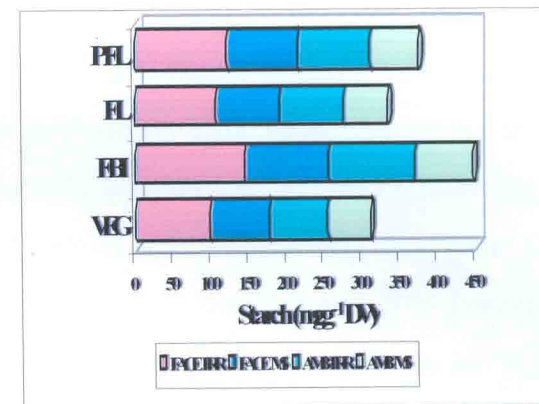
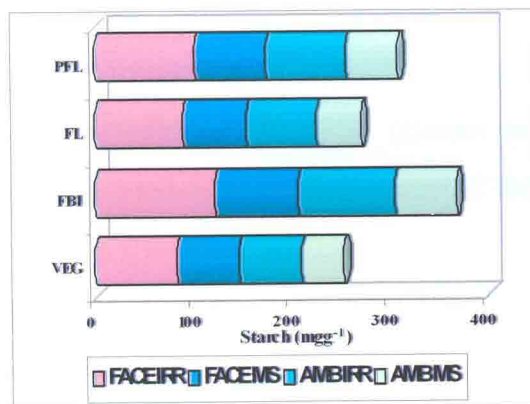
Elevated CO₂ significantly increased the total sugar content in roots of *Brassica* cultivars from 19% (vegetative) to 31% (flower bud initiation) (Fig. 25). It was more in 'RH-30' cultivar. Moisture stress treatment significantly decreased it and the reduction ranged between 31% (vegetative) to 52% (flower bud initiation). This reduction under ambient and elevated conditions varied from 24% (vegetative) to 38% (flower bud initiation) from 17% (vegetative) to 27% (post flowering), respectively in 'Pusa Gold' and between 22% (vegetative) to 37% (post flowering) and 16% (vegetative) to 22% (post flowering), respectively in 'RH-30'.

Leaf starch:

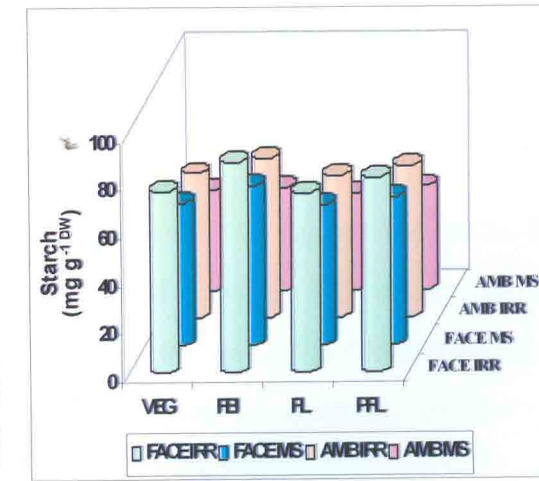
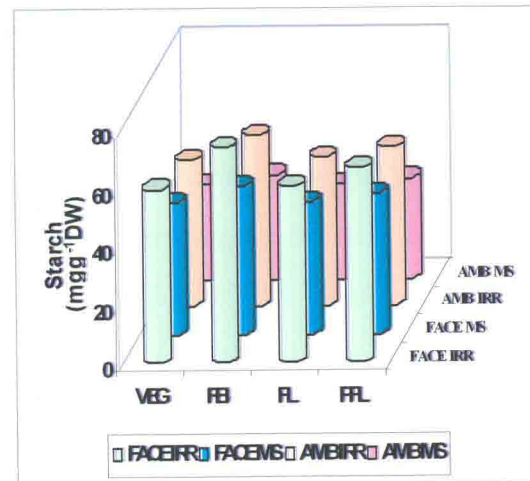
CO₂ enrichment significantly increased the starch content varying from 29% (vegetative) to 50% (flowering) (Fig. 26). Starch content was more in 'RH-30'. Moisture stress treatment significantly decreased it varying from 36% (vegetative)



Leaves



Stem



Pusa Gold

Roots

RH-30

Fig. 26 Interactive effect of elevated CO₂ and moisture stress on starch content in leaves, stem and roots of *Brassica* cultivars

to 48% (flowering). The reduction under ambient and elevated CO₂ conditions varied from 25% (vegetative) to 39% (flowering) and from 21% (vegetative) to 30% (post flowering), respectively in 'Pusa Gold' and between 28% (post flowering) to 39% (flowering) and from 20% (vegetative) to 27% (flowering), respectively in 'RH-30'.

Stem starch:

Elevated CO₂ significantly increase the starch content in stem throughout the growth period. Starch was more in the stem of 'RH-30' (Fig. 26). Moisture stress brought about significant reduction in the starch content varying between 35% (vegetative) to 48%, (flower bud initiation). This reduction under ambient and elevated CO₂ condition varied from 33% (vegetative) to 36% (flowering) and 23% (vegetative) to 28% (flower bud initiation), respectively in 'Pusa Gold', whereas, between 26% (vegetative) to 33% (post flowering) and 20% (vegetative) to 22% (flower bud initiation, flowering, post flowering), respectively in 'RH-30'.

Root starch:

CO₂ enrichment brought about significant increase in the starch content ranging from 28% (vegetative) to 35% (flower bud initiation) (Fig. 26). Starch content was more in the roots of 'RH-30' cultivar. Moisture stress treatment markedly decreased the starch content ranging from 43% (vegetative), 54% (flower bud initiation). This reduction under ambient and elevated CO₂ conditions varied from 35% (vegetative) to 39% (flower bud initiation) and 23% (vegetative) to 30% (flower bud initiation), respectively in 'Pusa Gold', whereas, between 30% (vegetative) to 36% (flower bud initiation) and 21% (vegetative) to 24% (flower bud initiation), respectively in 'RH-30'.

Non-structural carbohydrate (NSC) content at different parts:

Leaf

CO₂ enrichment significantly increased the non-structural carbohydrate content in leaves of *Brassica* cultivars ranging from 21% (vegetative) to 26%

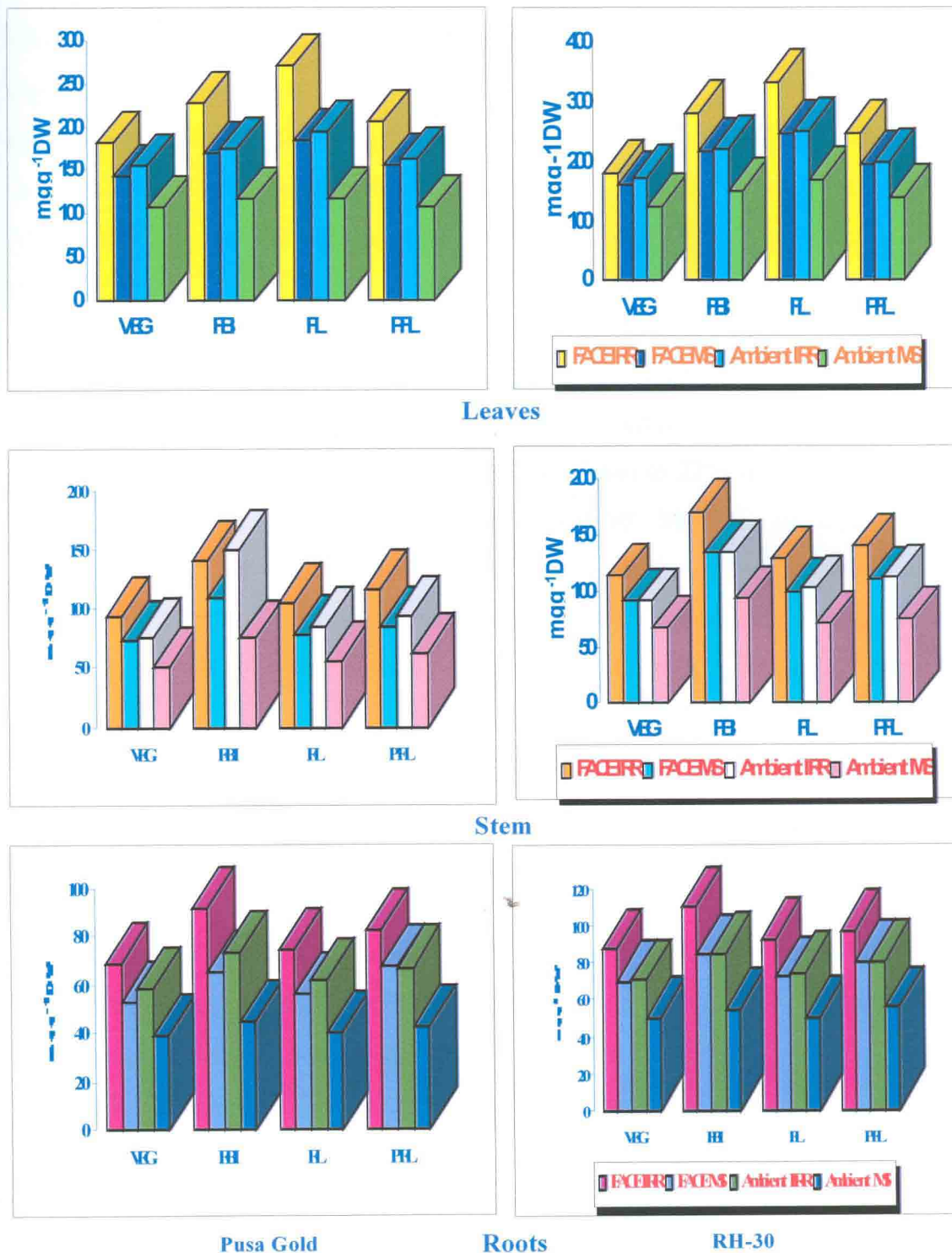


Fig: 27 Interactive effect of elevated CO₂ and moisture stress on non-structural carbohydrate in the leaves, stem and roots of *Brassica* cultivars

(flowering) (Fig. 27). NSC was more in 'RH-30'. Moisture stress brought about significant reduction in the non-structural carbohydrate ranging between 26% (vegetative) to 45% (flowering). This reduction under ambient and elevated CO_2 varied between 31% (vegetative) to 39% (flowering) and 20% (vegetative) to 31% (flowering), respectively in 'Pusa Gold', whereas, it varied between 29% (vegetative) to 32% (flowering) and 11% (vegetative) to 25% (flowering), respectively in 'RH-30'.

Stem

Elevated CO_2 markedly increased the non-structural carbohydrate of the shoot ranging between 22% (flowering bud initiation) to 32% (post flowering) (Fig. 27). This carbohydrates content was more in 'RH-30'. Moisture stress significantly decreased the non-structural carbohydrate ranging from 34% (vegetative) to 45% (flowering). This reduction under ambient and elevated conditions varied between 32% (vegetative) to 50% (flower bud initiation) and 22% (vegetative) to 26% (flowering), respectively in 'Pusa Gold' and between 27% (vegetative) to 32% (post flowering) and 20% (vegetative) to 22% (flowering), respectively in 'RH-30'.

Root

CO_2 enrichment significantly increased the non-structural carbohydrate in roots ranging between 28% (vegetative) to 33% (post flowering). This carbohydrate content was more in 'RH-30' (Fig. 27). Moisture stress significantly decreased the non-structural carbohydrate content ranging between (27% flowering) to 44% (flower bud initiation). This reduction under ambient and elevated CO_2 condition varied between 33% (vegetative) to 39% (post flowering) and 17% (post flowering) to 29% (flower bud initiation), respectively in 'Pusa Gold' and 29% (vegetative) to 35% (flower bud initiation) and 20% (vegetative to flower bud initiation), respectively in 'RH-30'.

Carbon content in different parts:

Leaf

The carbon content in the leaves of *Brassica* cultivars was significantly more

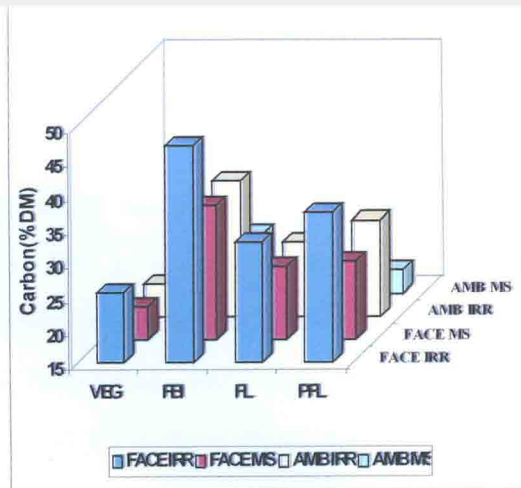
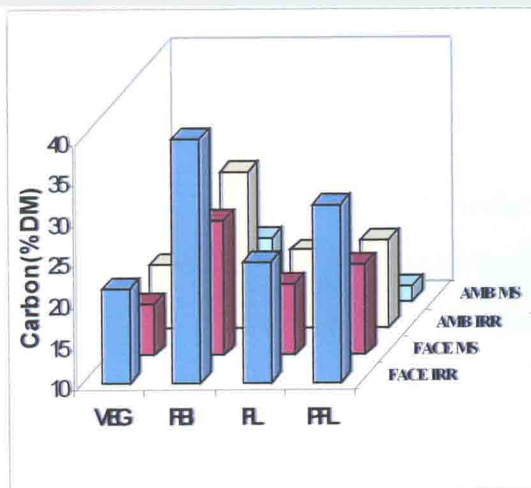
in plants grown under high CO₂ (Fig. 28). This increase ranged from 35% (vegetative) to 38% (flowering). Carbon content was more in the leaf of 'RH-30'. Moisture stress treatment significantly decreased the carbon content ranging from 47% (vegetative) to 60% (flowering and post flowering). The reduction under ambient and elevated CO₂ conditions varied between 37% (flowering) to 43% (post flowering) and 23% (vegetative) to 34% (flower bud initiation), respectively in 'Pusa Gold', whereas, in the case of 'RH-30' the reduction ranged between 31% (flowering) to 38% (vegetative) under ambient condition and between 21% (vegetative) to 28% (post flowering) under elevated CO₂ condition.

Stem

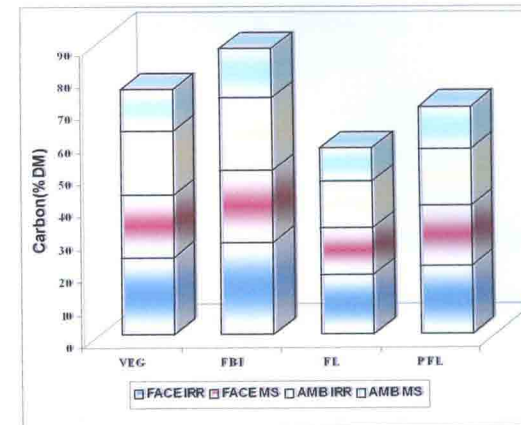
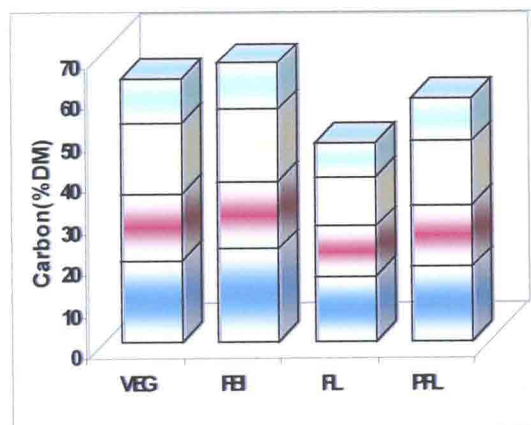
CO₂ enrichment significantly increased the carbon content between 28% (vegetative) to 38% (flower bud initiation) in the stem of *Brassica* cultivars (Fig. 28). Carbon content was more in the stem of 'RH-30'. Moisture stress significantly decreased it ranging between 35% (vegetative) to 45% (flower bud initiation). This reduction under ambient and elevated CO₂ condition varied between 30% (post flowering) to 36% (vegetative) and 15% (post flowering) to 26% (flower bud initiation), respectively in 'Pusa Gold', whereas, it ranged between 24% (post flowering) to 33% (vegetative) and 10% (vegetative) to 23% (flowering), respectively in 'RH-30'.

Root

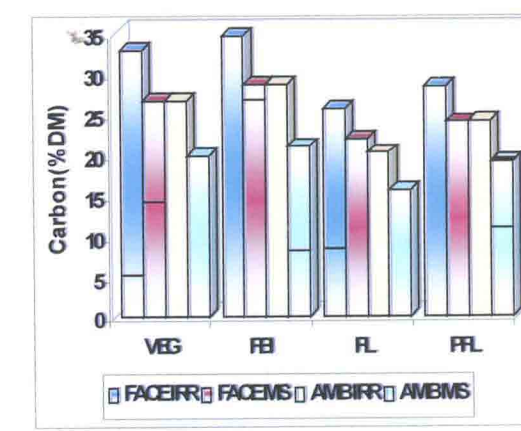
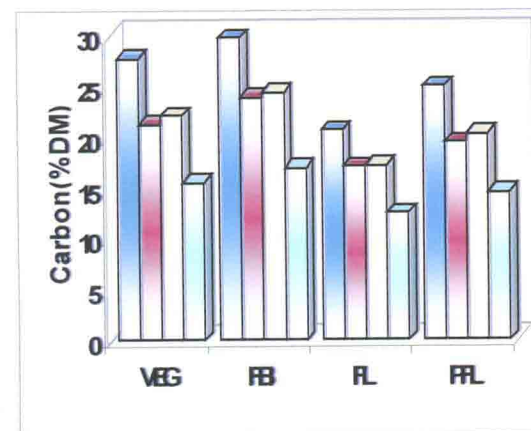
Elevated CO₂ markedly increased the carbon content of roots varying from 22% (flower bud initiation) to 32% (vegetative) (Fig. 28). Carbon content was more in roots of 'RH-30' cultivar. Moisture stress treatment significantly decreased the carbon in the roots between 33% (post flowering) to 41% (flower bud initiation). This reduction under ambient and elevated CO₂ condition varied between 27% (flowering) to 31% (flower bud initiation) and 17% (flowering) to 23% (vegetative), respectively in 'Pusa Gold' cultivar, whereas, between 21% (post flowering) to 26% (flower bud initiation) and 14% (flowering) to 19% (vegetative), respectively in 'RH-30'.



Leaves



Stem



Pusa Gold

Roots

RH-30

Fig. 28 Interactive effect of elevated CO₂ and moisture stress on carbon content in leaves, stem and roots of *Brassica* cultivars

Nitrogen content of the different parts:

Leaf

CO₂ enrichment significantly decreased the nitrogen content ranging from 12% (vegetative) to 29% (flowering) (Fig 29). It was higher in 'RH-30' compared to 'Pusa Gold'. Moisture stress significantly reduced the nitrogen content ranging between 19% (vegetative) to 24% post flowering. This reduction under ambient condition varied between 21% (flowering) to 28% (post flowering) in 'Pusa Gold', whereas, in 'RH-30', it ranged from 19% (flowering) to 27% (post flowering), respectively. The interactive effect of CO₂ and moisture stress on nitrogen was not significant.

Stem

CO₂ enrichment markedly decreased the nitrogen content ranging from 17% (vegetative) to 26% (flowering) (Fig. 29). Nitrogen content of stem was more in stem of 'RH-30'. Moisture stress decreased it significantly between 22% (post flowering) to 27% (flowering). The interactive effect of moisture stress and CO₂ on nitrogen content was not significant.

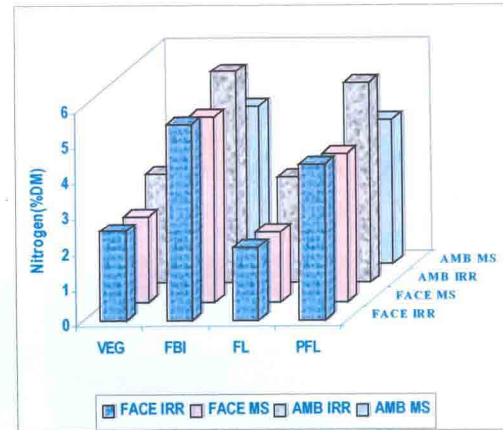
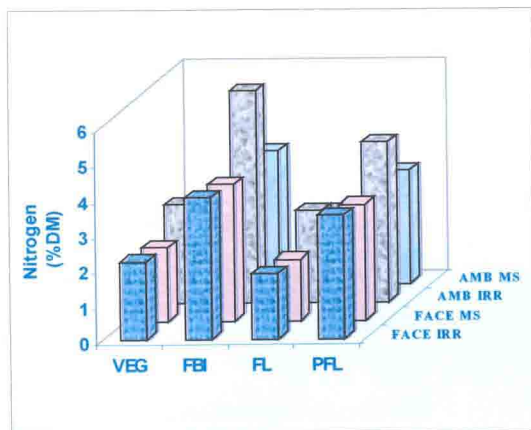
Root

Nitrogen content in roots of *Brassica* cultivars was significantly decreased due to CO₂ enrichment varying from 12% (flower bud initiation) 16% (flowering) (Fig. 29). Nitrogen content was more in roots of 'RH-30'. Moisture stress significantly reduced it ranging from 18% (flower bud initiation) to 22% (flowering). The interactive effect of moisture stress and CO₂ on nitrogen content was not significant.

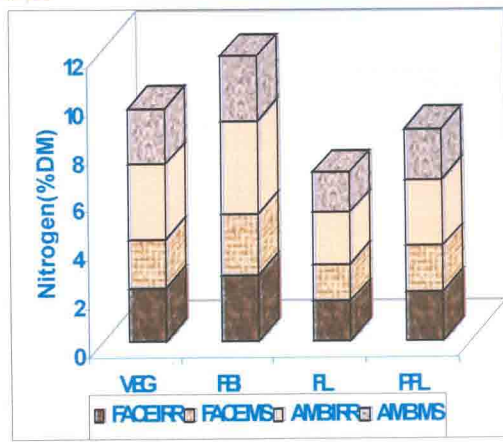
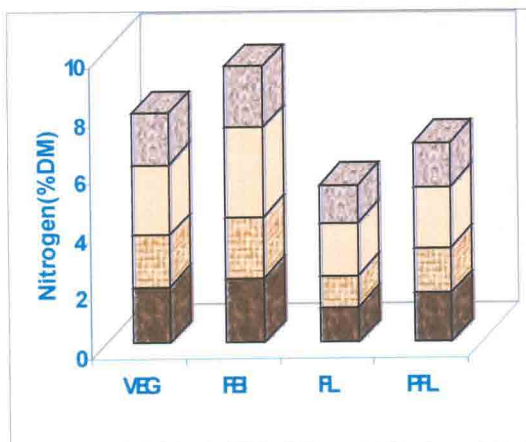
C/N ratio of different parts of plant:

Leaves

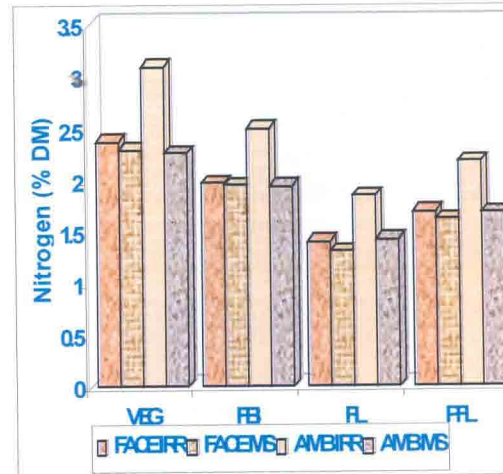
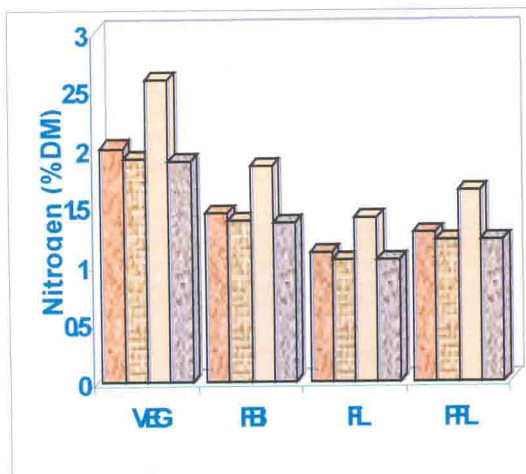
CO₂ enrichment brought about significant increase in the C/N ratio in leaves ranging between 51% (Post flowering) to 72% (flower bud initiation) (Fig. 30).



Leaves



Stem



Pusa Gold

Root

RH-30

Fig. 29 Interactive effect of elevated CO₂ and moisture stress on nitrogen content in leaves, stem and root of *Brassica* cultivars

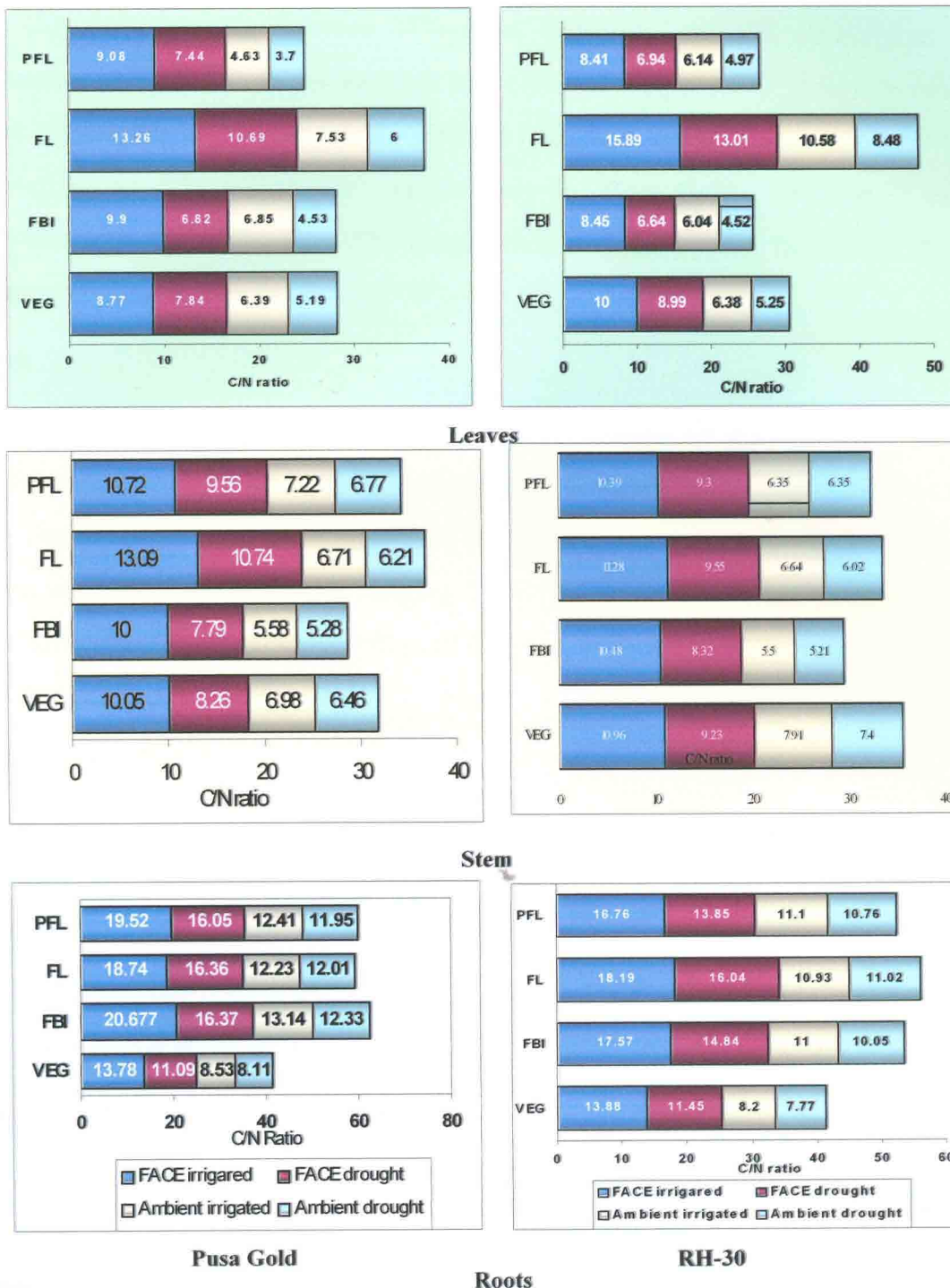


Fig. 30 Interactive effect of elevated CO₂ and moisture stress on C/N ratio of leaves, stem and roots of *Brassica* cultivars

Varietal difference for this character was not significant. Moisture stress reduced the C/N ratio ranging between 11% (post flowering) to 34% (flowering). The reduction in C/N ratio under ambient and elevated CO₂ conditions varied between 20% (flowering and flower bud initiation) to 34% (vegetative) and 17% (post flowering) to 31% (vegetative), respectively in 'Pusa Gold', whereas, it ranged between 19% (flowering) to 20% (vegetative) and 15% (post flowering) to 21% (vegetative), respectively in 'RH-30'.

Stem

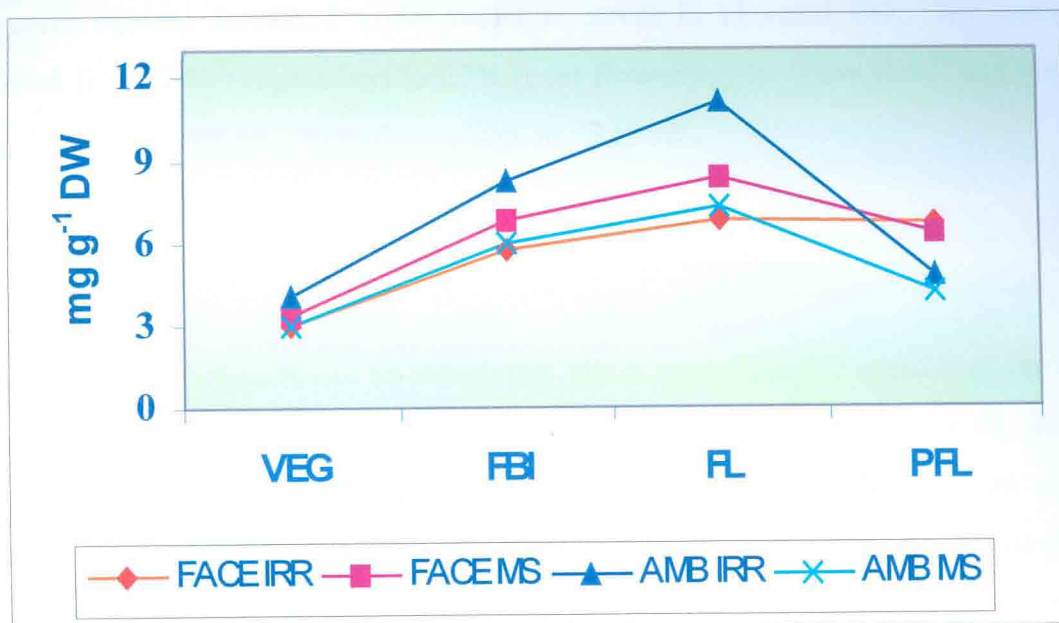
Elevated CO₂ brought about significant increase in the C/N ratio in stem of *Brassica* ranging from 48% (post flowering) to 67% (flower bud initiation) (Fig. 30). There was no significant difference in C/N ratio between the cultivars. Moisture stress decreased the C/N ratio ranging from 10% (post flowering) to 21% (flower bud initiation). The interactive effect of CO₂ and moisture stress was not significant for this character.

Root

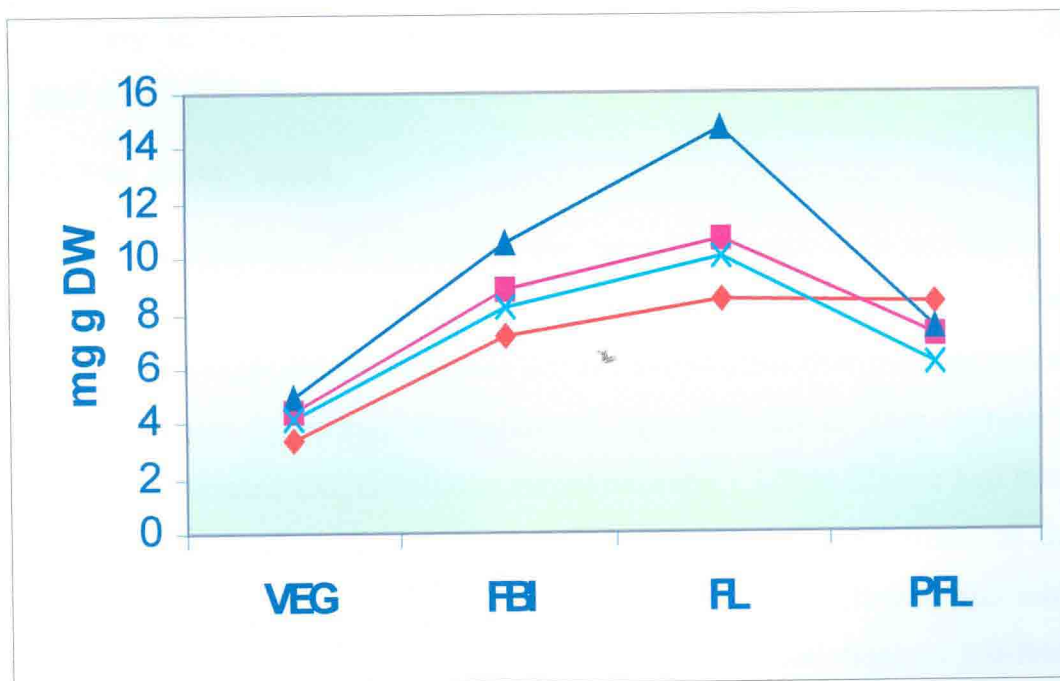
Elevated CO₂ brought about significant increase the C/N ratio ranging between 20% (flower bud initiation) to 52% vegetative) (Fig. 30). There was no significant vertical difference in C/N ratio. Moisture stress significantly decreased it from 11% (vegetative stage) to 46% (flower bud initiation). The interactive effect of CO₂ and moisture stress was not significant for these characters.

Total soluble protein in leaves:

CO₂ enrichment significantly decreased the soluble protein in the leaves of *Brassica* cultivars ranging from 14%(vegetative) to 26% (flowering) (Fig 31). Soluble protein content was comparatively more in 'RH-30' cultivar. Moisture stress had no significant effect on soluble protein in leaves. The stress induced reduction on protein content varied between 24% (vegetative) to 34% (flowering) in 'Pusa Gold', whereas, in 'RH-30', 17% (post flowering) to 32% (flowering) under ambient condition. Complete reverse trend was observed under elevated CO₂.



Pusa Gold



RH-30

Fig. 31 Interactive effect of elevated CO₂ and moisture stress on leaf soluble protein content in *Brassica* cultivars

Protein content increased under moisture stress in elevated CO₂. The increment varied from 13% (vegetative) to 27% (post flowering) in 'Pusa Gold' and between 17% (vegetative) to 27% (flowering) in 'RH-30'.

Osmotic adjustment:

Proline

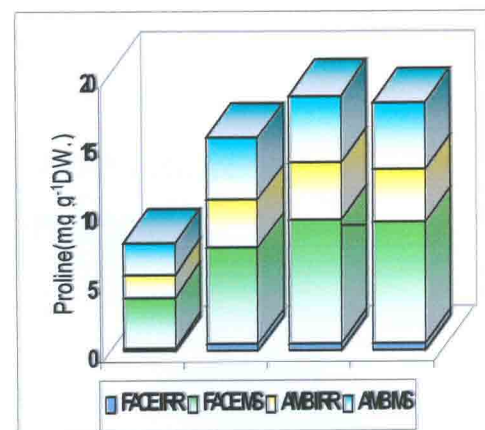
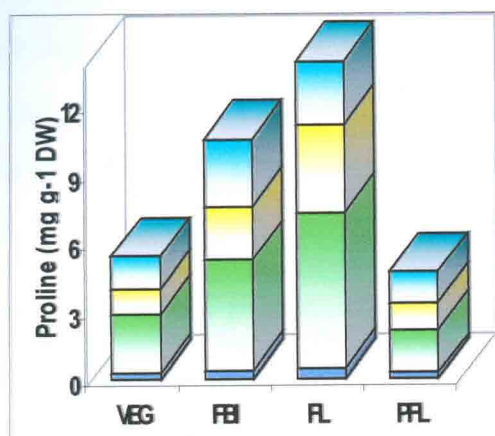
CO₂ enrichment has no significant effect on the proline content of the leaves of *Brassica* cultivar (Fig. 32). Proline content was more in leaves of 'RH-30'. Moisture stress significantly increased the proline content ranging from 2.2-fold (vegetative), to 2.7-fold (flowering). The stress induced increase in proline content under ambient and elevated CO₂ conditions ranged from 1.1-fold (flowering) to 1.3-fold (vegetative) and 7.4-fold (post flowering) to 14.0-fold (flowering), respectively in 'Pusa Gold', whereas, it was 1.3-fold and 11.9-fold (vegetative) to 15.7-fold (post flowering), respectively in 'RH-30'.

Total free amino acids

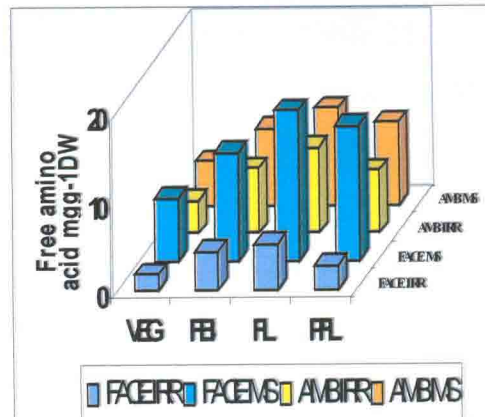
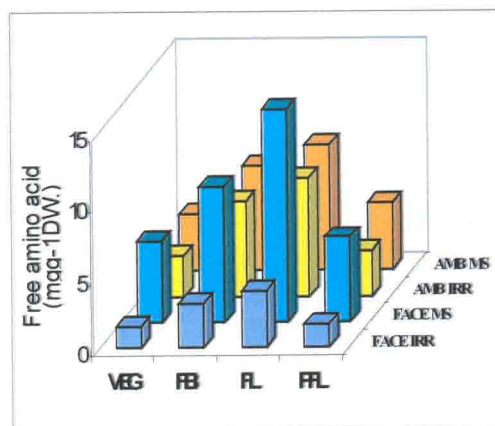
CO₂ enrichment did not affect the total free amino acid content in leaves of *Brassica* (Fig. 32). Free amino acids were higher in 'RH-30' cultivar. Moisture stress treatment significantly increased the free amino acids from 1.6-fold (vegetative) to 2.3-fold (post flowering) throughout the growth period. This increase under ambient and elevated CO₂ conditions varied between 1.1-fold (flower bud initiation) to 1.6-fold (post flowering) and 3.0-fold (flower bud initiation) to 3.9-fold (vegetative), respectively in 'Pusa Gold' and from 1.2-fold (flower bud initiation) to 1.6-fold (post flowering) and 2.9-fold (flower bud initiation) to 6.0-fold (post flowering), respectively in 'RH-30'.

Total free fatty acids:

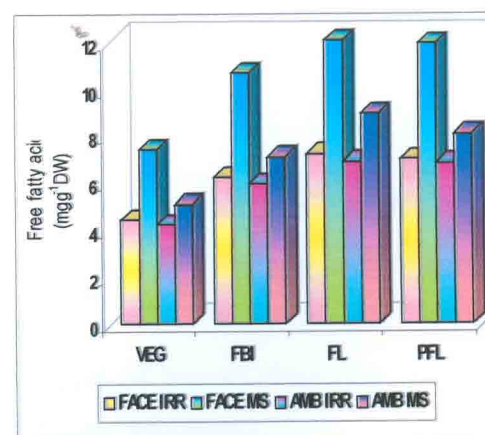
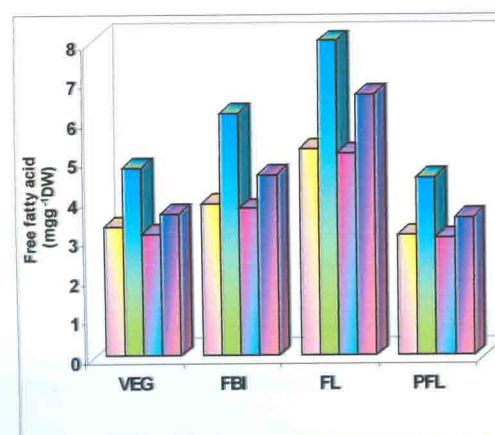
CO₂ enrichment significantly increased the free fatty acids content in leaves of *Brassica* ranging between 24-26% (Fig. 32). The free fatty acids content was more in leaves of 'RH-30' compared to 'Pusa Gold'. Moisture stress significantly



Proline



Free amino acid



Pusa Gold

Free fatty acid

RH-30

Fig 32 Interactive effect of elevated CO₂ and moisture stress on proline, free amino acid and free fatty acid of *Brassica* cultivars.

increased the free fatty acid content ranging between 39% (vegetative) to 54% (flowering). The stress induced increase on free fatty acids under ambient and elevated CO₂ varied between 14% (vegetative) to 29% (flowering) and 46% (vegetative) to 75% (flowering), respectively in 'Pusa Gold', whereas, this increase ranged between 18% (post flowering) to 30% (flowering) and 67% (vegetative) to 79% (flowering), respectively in 'RH-30'.

Water relation parameters:

Proton spin-lattice relaxation time (T₁)

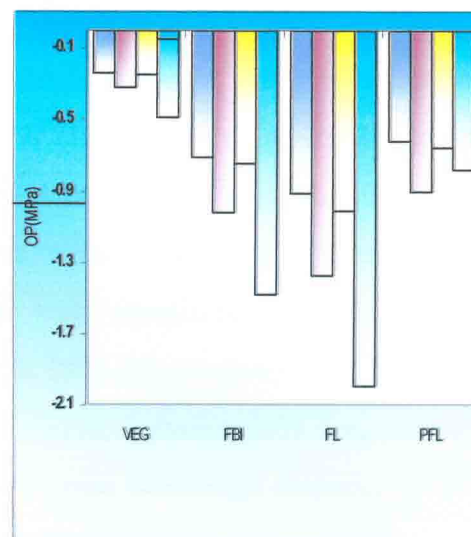
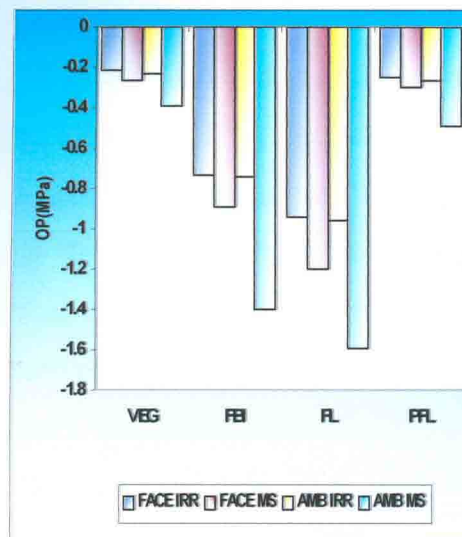
The elevated level of CO₂ brought about significant increase in the proton spin-lattice relaxation time in leaves of *Brassica* cultivars (Table 25). This increase ranged between 26% (vegetative) to 31% (flower bud appearance). Proton spin-lattice relaxation time was significantly more in 'RH-30' compared to 'Pusa Gold'. Moisture stress brought about significant reduction in proton spin-lattice relaxation time from 28% (vegetative) to 43% (flowering). This reduction under ambient and elevated CO₂ condition varied between 24% (vegetative) to 34% (flowering) and 16% (vegetative) to 20% (flowering), respectively in 'Pusa Gold' and between 27% (vegetative) to 31% (post flowering) and 13% (post flowering) to 18% (flower bud initiation), respectively in 'RH-30'.

Osmotic potential (OP)

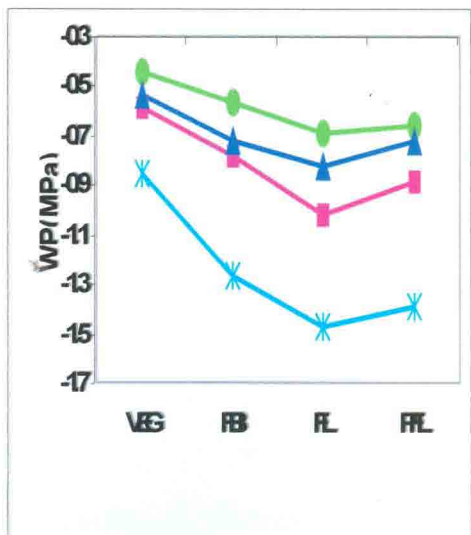
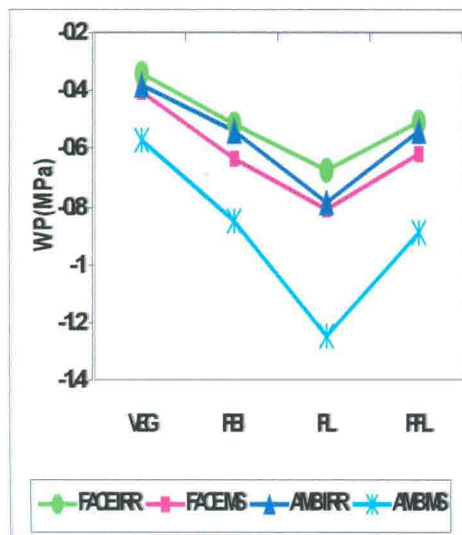
CO₂ enrichment significantly enhanced the osmotic potential in leaves of *Brassica* cultivars between 22% (flower bud initiation) to 54% (post flowering) (Fig. 33). Osmotic potential was more in 'RH-30'. Moisture stress treatment significantly decreased the osmotic potential of leaves. The reduction varied from 37% (post flowering) to 70% (flowering). The stress-induced reduction under ambient and elevated CO₂ conditions varied between 48% (vegetative) to 62% (flowering) and 25% (vegetative) to 33% (flowering), respectively in 'Pusa Gold', whereas, in 'RH-30' it ranged between 35% (flower bud initiation) to 45% (post flowering) and 16% (flowering) to 20% (post flowering), respectively.

Table 25 Interactive effect of elevated CO₂ and moisture stress on proton pulse relaxation time (T₁, ms) in leaf different stages of growth

Treatments	Vegetative		Flower bud initiation		Flowering		Post flowering	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	334	390	400	460	410	580	330	520
FACE moisture stress	279	326	320	375	327	480	277	450
Ambient irrigated	283	332	335	380	340	485	280	451
Ambient moisture stress	204	240	220	261	222	342	202	339
CV.	32.43		16.42		59.34		59.23	
CO ₂	23.44		8.31		27.44		30.44	
CV. x CO ₂	47.54		11.76		49.33		49.21	
MS	23.76		8.09		50.02		26.67	
CV. x MS	44.36		11.44		66.32		42.89	
CO ₂ x MS	56.32		13.67		78.11		54.56	
CV. x CO ₂ x MS	67.44		17.89		87.44		68.49	



Osmotic potential



Pusa Gold

Water potential

RH-30

Fig. 33 Interactive effect of elevated CO₂ and moisture stress on osmotic and water potential of *Brassica* cultivars

Water potential (WP)

The elevated CO₂ caused significant increase in water potential of leaves ranging between 31% (vegetative) to 37% (flowering) (Fig. 33). Water potential was more in the leaves of 'RH-30'. Moisture stress significantly decreased it ranging from 33% (Post flowering) to 61% (flowering). The stress-induced reduction under ambient and elevated CO₂ conditions varied between 37% (vegetative) to 48% (post flowering) and 25% (vegetative) to 27% (flower bud initiation), respectively in 'Pusa Gold', whereas, between 33% (vegetative) to 39% (post flowering) and 16% (flowering) to 18% (post flowering), respectively in 'RH-30'.

Leaf water content (RWC)

The elevated CO₂ brought about significant increase in RWC in the leaves of *Brassica* species ranging from 19% (post flowering) to 23% (flowering) (Table 26). RWC was more in the leaves of 'RH-30'. Moisture stress significantly depressed the RWC between 25% (vegetative) to 40% (post flowering). The stress induced decrease under ambient condition in 'Pusa Gold' varied from 29% (vegetative) to 67% (post flowering), where as, in 'RH-30' it ranges between 21% (post flowering) to 52% (post flowering). There was no significant difference under elevated CO₂. The interactive effect of CO₂ and moisture stress was not significant.

Antioxidant enzymes:

Super oxide dismutase (SOD)

The increased concentration of CO₂ significantly decreased the SOD activity in leaves and the reduction ranged from 1.1-fold (vegetative) to 1.3-fold (flowering) (Fig. 34). SOD activity was higher in 'RH-30' cultivar. Moisture stress significantly increased the SOD activity between 2.2-fold (flowering) to 2.5-fold (Post flowering). This increase under ambient and elevated CO₂ condition varied between 1.1-fold (post flowering) to 1.3-fold (flower bud initiation) and 5.3-fold (flower bud initiation) to 7.3-fold (post flowering), respectively in 'Pusa Gold' cultivar, whereas, it ranged between 1.2-fold (vegetative) to 1.4-fold (flowering) and 5.9-fold (vegetative) to 10.2-fold (post flowering), respectively in 'RH-30'.

Table 26. Interactive effect of elevated CO₂ and moisture stress on relative leaf water content (%) different stages of growth

Treatments	Vegetative			Flower bud initiation			Flowering			Post flowering	
	Pusa Gold	RH-30	Pusa Gold	Pusa Gold	RH-30	Pusa Gold	Pusa Gold	RH-30	Pusa Gold	RH-30	RH-30
FACE irrigated	75.47	83.60	80.90	89.92	84.00	72.12	77.00	88.06	60.64	79.76	79.76
FACE moisture stress	71.57	76.90	74.00	84.00	84.00	72.12	72.12	80.60	56.02	72.86	72.86
Ambient irrigated	74.00	79.40	77.80	87.10	87.10	74.40	74.40	84.60	57.00	77.27	77.27
Ambient moisture stress	49.20	51.02	51.39	56.03	56.03	48.08	48.08	50.92	41.90	50.01	50.01
CV.	6.85		4.52			5.02				5.49	
CO ₂	6.49		6.65			5.50				4.79	
CV. x CO ₂	9.18		9.41			7.18				6.37	
MS	3.17		3.34			3.71				3.97	
CV. x MS	4.31		4.82			5.25				5.20	
CO ₂ x MS	6.89		7.14			7.42				7.94	
CV. x CO ₂ x MS	8.61		9.63			10.49				10.51	

Catalase (CAT)

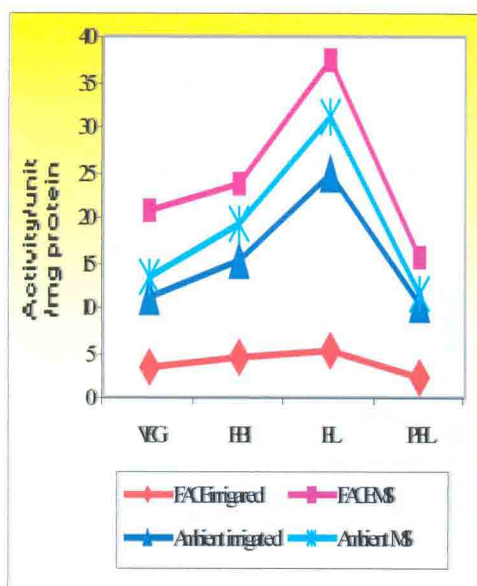
Higher level of CO₂ significantly decreased the CAT activity by 1.1-fold (vegetative) to 3.3-fold (post flowering) (Fig. 35). CAT activity was higher in the 'RH-30'. Moisture stress significantly increased it 2.0-fold (post flowering) to 2.4-fold (flower bud initiation). This enhancement under ambient and elevated CO₂ conditions was 1.1-fold (post flowering) to 1.3-fold (flower bud initiation) and between 2.3-fold (post flowering) to 7.2 fold (flowering), respectively in 'Pusa Gold', whereas, in the case of 'RH-30' the increment was 1.3-fold and 5.5 (post flowering) to 7.7-fold (post flowering), respectively.

Ascorbate peroxidase (APOX)

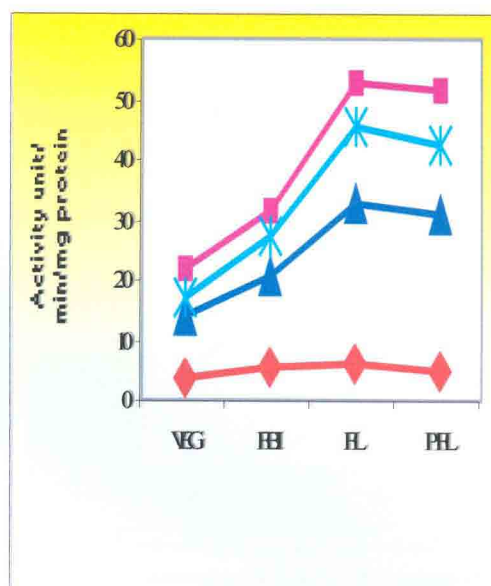
The elevated CO₂ significantly decreased the APOX activity between 1.2-fold (vegetative) to 2.1-fold (post flowering) (Fig. 36). APOX activity was higher in the 'RH-30'. Moisture stress significantly increased the APOX by 1.9-fold. This increase under ambient and elevated CO₂ condition varied between 1.1-fold (post flowering) to 1.3-fold (flower bud initiation) and 5.3-fold (flower bud initiation) to 7.3-fold (post flowering), respectively in 'Pusa Gold' and between 1.2 fold (vegetative) to 1.4 fold (flowering) and 5.9-fold (vegetative) to 10.2-fold (post flowering) in 'RH-30', respectively.

Peroxidase (POX)

The elevated level CO₂ brought about significant reduction (1.2-fold) in the POX activity (Fig. 37). POX activity was higher in 'RH-30'. Moisture stress increased the POX activity between 2.3-fold (vegetative) to 2.6-fold (flowering). This increase under ambient and elevated CO₂ condition was 1.3-fold and between 5.7-fold (vegetative) to 7.3-fold (post flowering), respectively in 'Pusa Gold', whereas, in the case of 'RH-30' the increment ranged between 1.3-fold (vegetative) to 1.5-fold (flowering) and 6.0-fold (vegetative) to 8.7-fold (post flowering), respectively

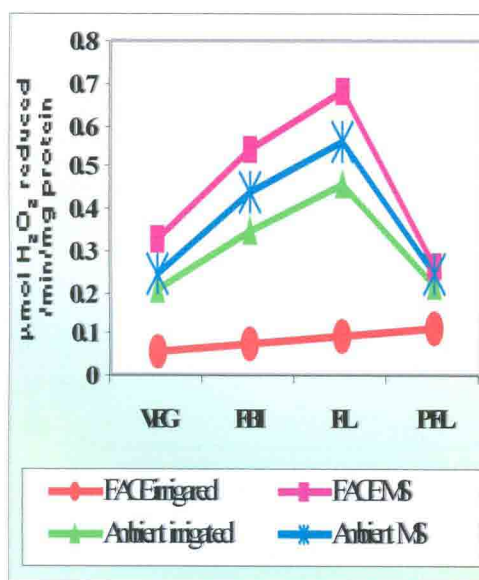


Pusa Gold

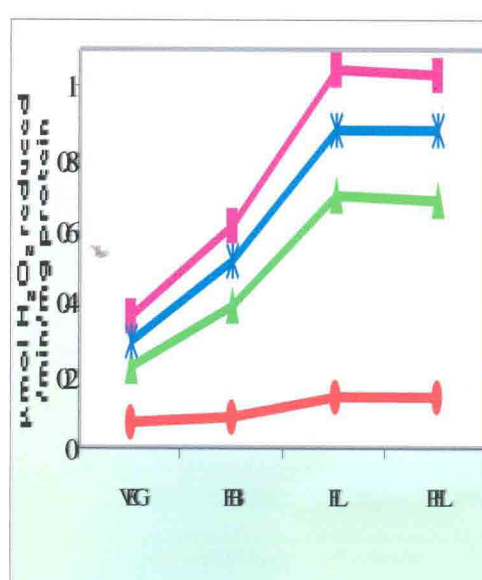


RH-30

Fig. 34 Interactive effect of elevated CO₂ and moisture stress on superoxide dismutase activity in *Brassica* cultivars



Pusa Gold



RH-30

Fig. 35 Interactive effect of elevated CO₂ and moisture stress on catalase activity in *Brassica* cultivars

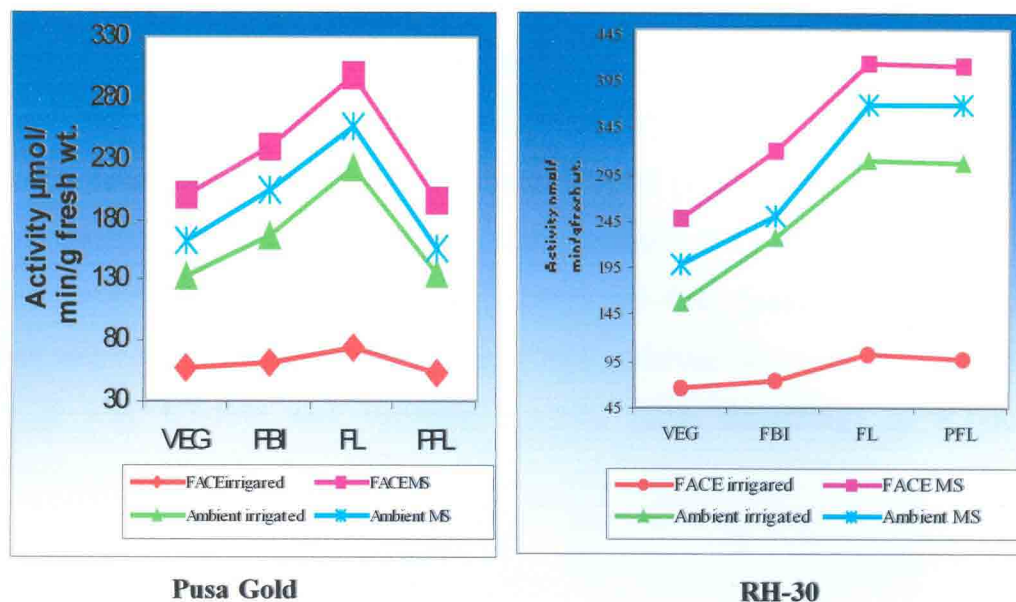


Fig. 36 Interactive effect of elevated CO₂ and moisture stress on ascorbate peroxidase activity in *Brassica* cultivars

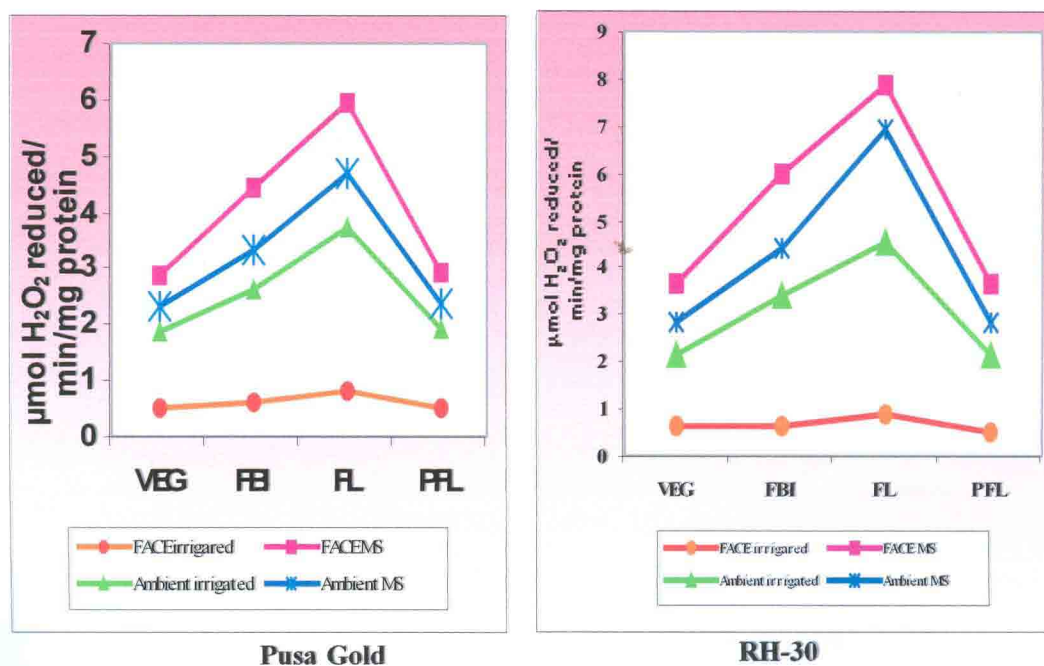


Fig. 37 Interactive effect of elevated CO₂ and moisture stress on peroxidase activity in *Brassica* cultivars

Glutathione reductase (GR)

Elevated CO₂ significantly decreased the GR activity from 1.1-fold (vegetative) to 1.7-fold (post flowering) (Fig. 38). GR activity was higher of 'RH-30'. Moisture stress increased the GR activity between 2.3- fold (vegetative), 2.6-fold (flowering). This enhancement under ambient and elevated CO₂ condition was 1.3- fold throughout the growth period and between 4.1-fold (post flowering) to 8.2-fold (post vegetative), respectively in 'Pusa Gold' cultivar, whereas, in the case of 'RH-30' it was 1.3-fold and between 3.4-fold (post flowering) to 6.1-fold (post flowering), respectively.

Membrane related phenomenon:

Hydrogen peroxide (H₂O₂)

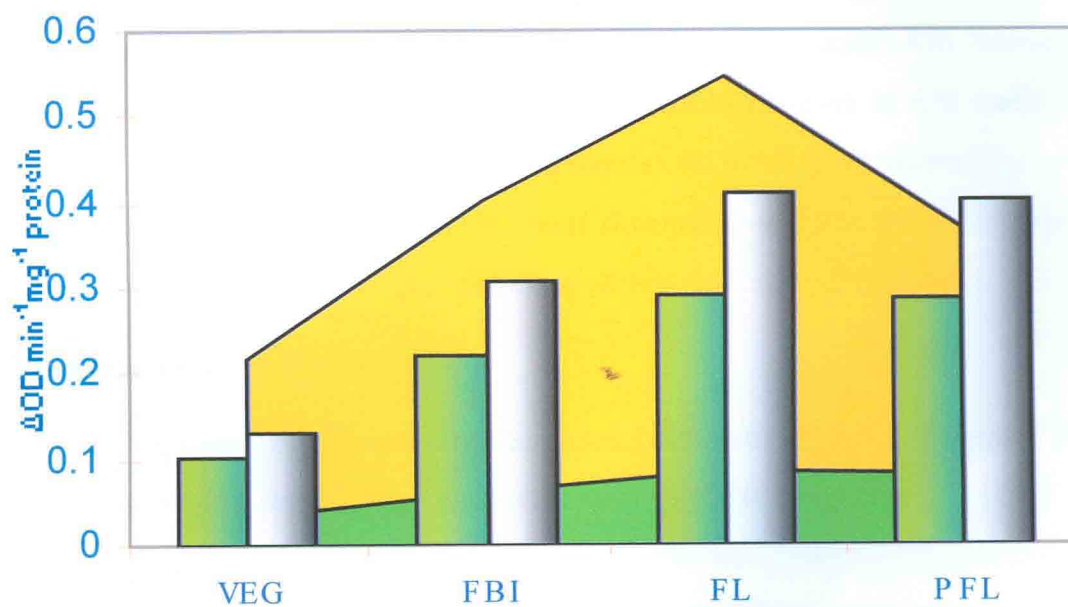
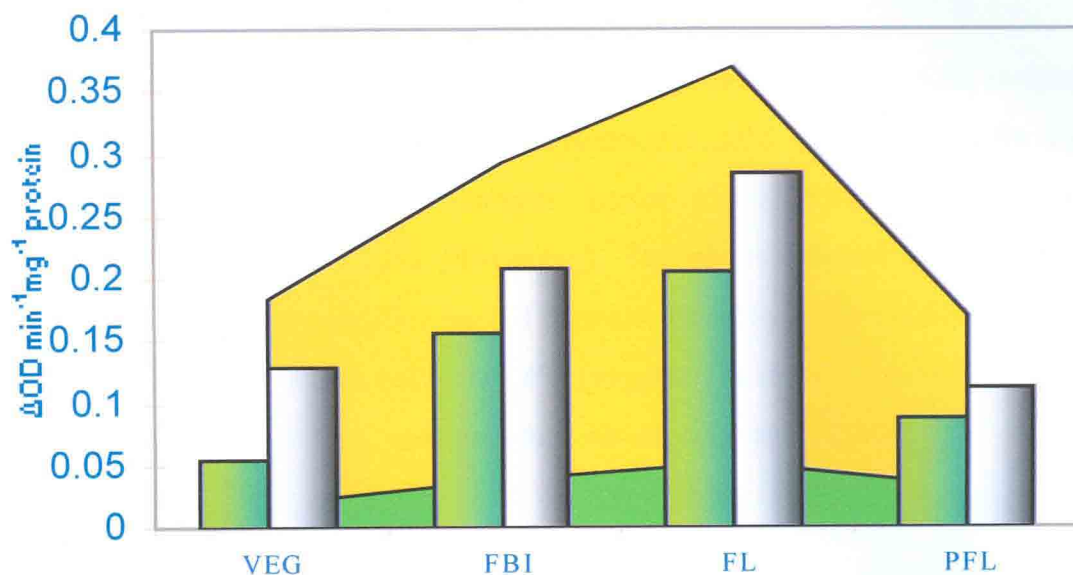
There was significant decrease in H₂O₂ content ranging from 20% (vegetative) to 41% (flowering) due to elevated CO₂ (Table 27). H₂O₂ content was significantly lower in 'RH-30'. Moisture stress increased the H₂O₂ content between 20% (vegetative) to 40% (post flowering). The stress induced increase in 'Pusa Gold' under ambient condition varied between 31% (vegetative) to 76% (post flowering), whereas, it ranged between 30% (vegetative) to 61% (post flowering) in 'RH-30'. The interactive effect of CO₂ and moisture stress was not significant.

Malondialdehyde content (MDA)

Elevated CO₂ brought about significant decrease in MDA content between 13% (vegetative) to 37% (flowering) (Table 28). MDA content of 'RH-30' cultivar was significantly lower as compared to 'Pusa Gold' throughout the growth period. Moisture stress caused to increase in MDA content between 14% (vegetative) to 38% (post flowering). The stress induced increase in 'Pusa Gold' under ambient condition varied between 27% (vegetative) to 72% (post flowering), whereas, it ranged between 18% (vegetative) to 60% (post flowering) in 'RH-30'. The interactive effect of CO₂ and moisture stress was not significant for this character.

Table 28. Interactive effect of elevated CO₂ and moisture stress on lipid peroxidation (nmol TBARS /g dry wt) at different stages of growth

Treatments	Vegetative		Flower bud initiation		Flowering		Post flowering	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	1.18	0.93	1.92	1.30	2.10	1.50	2.20	1.58
FACE moisture stress	1.24	0.98	1.98	1.39	2.27	1.62	2.39	1.84
Ambient irrigated	1.22	0.97	1.91	1.40	2.25	1.60	2.36	1.83
Ambient moisture stress	1.550	1.15	2.90	2.10	3.89	2.57	3.65	2.67
CV.	0.122		0.102		0.381		0.138	
CO ₂	0.848		0.067		0.265		0.299	
CV. x CO ₂	0.104		0.095		0.376		0.423	
MS	0.085		0.110		0.208		0.266	
CV. x MS	0.101		0.156		0.294		0.376	
CO ₂ x MS	0.111		0.221		0.416		0.532	
CV. x CO ₂ x MS	17.60		19.89		23.71		20.04	



■ FACE irrigated
 ■ FACE MS
 ■ Ambient irrigated
 ■ Ambient MS

RH-30

Fig. 38 Interactive effect of elevated CO₂ and moisture stress on glutathione reductase activity in *Brassica* Cultivars

Membrane stability index (MSI)

There was significant increase in MSI due to the elevated CO₂ ranging from 15% (vegetative) to 21% (flowering) (Table 29). MSI was significantly higher in 'RH-30'. Moisture stress brought about significant reduction in MSI from 17% (vegetative) to 22% (flowering). The stress-induced reduction in 'Pusa Gold' under ambient condition varied between 19% (vegetative) to 26% (flowering) and in 'RH-30' it ranged between 14% (vegetative) to 20% (post flowering). The interactive effect of CO₂ and moisture stress was not significant.

Relative stress injury (RSI)

Significant decrease in RSI was observed due to the CO₂ treatment ranging between 15% (vegetative) to 30% (post flowering) (Table 30). RSI was significantly lesser in the leaves of 'RH-30'. Moisture stress increased RSI between 16% (vegetative) to 43% (flowering). The stress induced increase in RSI under ambient in 'Pusa Gold' varied from 29% (vegetative) to 67% (post flowering), whereas, in 'RH-30' it ranges between 21% (post flowering) to 52% (post flowering). The interactive effect of CO₂ and moisture stress was not significant.

Lipoxxygenase (LOX) activity

CO₂ enrichment markedly decreased LOX activity in the leaves of *Brassica* cultivars ranging from 18% (vegetative) to 35% (flowering) (Fig. 39). LOX activity was lower in 'RH-30'. Moisture stress increased the LOX activity ranging between 20% (vegetative) to 37% (flowering). The stress induced increase in LOX activity under ambient condition varied from 34% (vegetative) to 70% (post flowering) in 'Pusa Gold', whereas, in 'RH-30' it ranged between 29% (vegetative) to 57% (flowering). The interactive effect of CO₂ and moisture stress was not significant.

Senescence intensity (SI)

There was marked decrease in SI due to the CO₂ treatment ranging between 17% (vegetative) to 30% (flowering) (Fig. 40). SI was lower in the leaves of 'RH-30' cultivar. Moisture stress significantly increased the SI ranging between 13%

Table 29. Interactive effect of elevated CO₂ and moisture stress on membrane stability index (%) at different stages of growth

Treatments	Vegetative			Flower bud initiation			Flowering			Post flowering		
	Pusa Gold	RH-30		Pusa Gold	RH-30		Pusa Gold	RH-30		Pusa Gold	RH-30	
FACE irrigated	76.40	85.64		78.01	87.84		78.00	91.64		63.19	77.24	
FACE moisture stress	70.25	79.18		71.64	81.70		72.54	84.17		58.10	71.12	
Ambient irrigated	71.36	80.22		73.00	83.85		73.54	85.05		59.00	72.26	
Ambient moisture stress	57.25	60.05		55.01	60.21		54.01	58.08		48.54	52.14	
CV.	4.36			4.01			2.76			4.99		
CO ₂	3.24			3.17			3.432			2.83		
CV. x CO ₂	5.55			4.48			4.85			3.99		
MS	3.59			3.77			2.98			3.42		
CV. x MS	4.24			4.50			3.81			4.84		
CO ₂ x MS	5.17			5.54			5.97			6.85		
CV. x CO ₂ x MS	6.98			7.00			7.61			9.68		

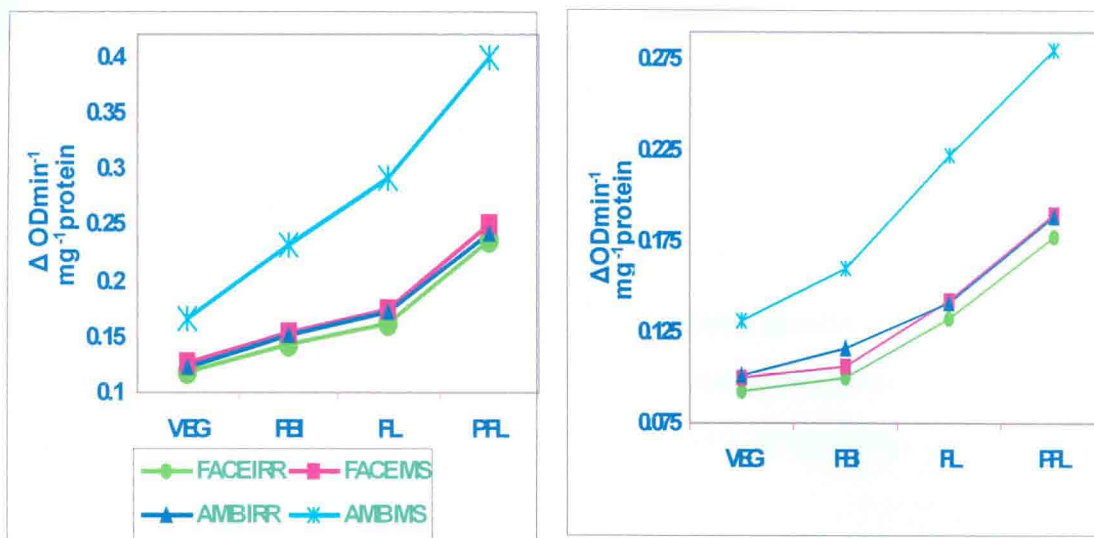


Fig. 39. Interactive effect of elevated CO₂ and moisture stress on lipoxygenase activity in *Brassica* cultivars.

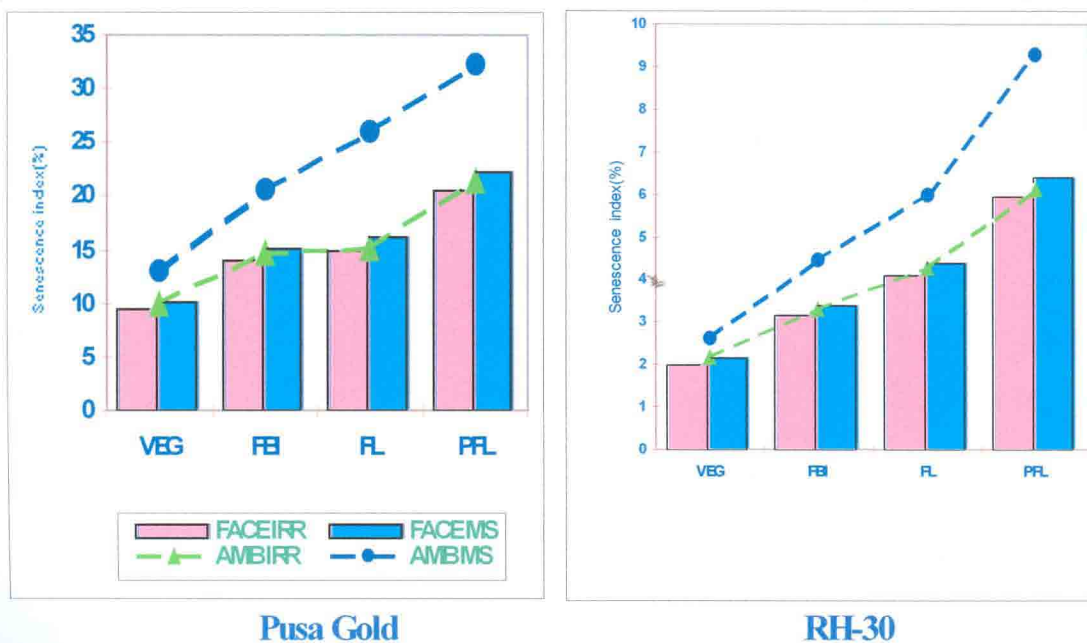


Fig. 40 Interactive effect of elevated CO₂ and moisture stress on senescence index in *Brassica* cultivars.

(vegetative) to 38% (flowering). The stress induced increase under ambient condition varied 30% (vegetative) to 72% (flowering) in 'Pusa Gold', whereas it ranged between 28% (vegetative) to 57% (flowering), in 'RH-30'. The interactive effect of CO₂ and moisture stress was not significant.

Anatomical characters:

To analyze the anatomic strategy of plants synchronizing with the rise in atmospheric CO₂ the leaves, stem, roots and internal structures were studied using section cutting TEM and other light microscopy technology.

Shoot anatomy

Vessel characters

Significant increase in different vessel characters was observed with elevated level of CO₂ (Table 31). Elevated CO₂ increased number of vessel by 24%, length by 28%, breadth by 15% and area by 43%. These characters were significantly greater in 'RH-30'. Moisture stress brought about marked reduction in vessel number (39%), length (33%), breadth (15%), and in area (77%). The stress-induced reduction under ambient and elevated conditions for these parameters was 38% for vessel number, 29% for length, 31% for breadth and 44% for area and 20% for number, 20% for length, 19% for breadth and 35% for area, respectively in 'Pusa Gold', whereas, in 'RH-30' this reduction was 35% for vessel number, 23% for length, 28% for breadth and 44% for area and 16% for number, 15% for length, 16% for breadth and 28% for area, respectively.

Phloem characters

The phloem number (31%), length (36%) breadth (38%) and area (82%) increased due to elevated CO₂ (Table 32). These phloem characters were significantly better in 'RH-30'. Moisture stress brought about marked reduction in the number of phloem (31%) length (42%) breadth (42%) and area (96%). The reduction on these parameters of phloem under ambient and elevated CO₂ conditions was 28% (number), 33% (length), 42% breadth and 60% area, respectively in 'Pusa Gold',

Table 31. Interactive effect of CO₂ and moisture stress on vessel characters of shoot

Treatments	Pusa Gold				RH-30			
	Number	Length (μm)	Breadth (μm)	Area (μm^2)	Number	Length (μm)	Breadth (μm)	Area (μm^2)
FACE irrigated	15.0	11.50	13.10	150.65	19.00	14.20	15.90	225.78
FACE moisture stress	12.0	9.20	10.60	97.52	16.00	12.10	13.40	162.08
Ambient irrigated	13.00	9.40	10.80	101.52	17.00	12.20	16.20	197.64
Ambient moisture stress	8.00	6.70	7.40	49.58	11.00	9.40	11.70	109.98
CV.	0.87	0.62	0.32	3.99	0.87	0.62	0.32	3.99
CO ₂	1.21	0.83	0.44	7.96	1.21	0.83	0.44	7.96
CV. x CO ₂	1.98	1.17	0.63	11.26	1.98	1.17	0.63	11.26
MS	0.76	0.31	0.43	1.97	0.76	0.31	0.43	1.97
CV. x MS	0.84	0.43	0.61	2.78	0.84	0.43	0.61	2.78
CO ₂ x MS	1.24	0.61	0.72	3.01	1.24	0.61	0.72	3.01
CV x CO ₂ x MS	2.12	1.26	0.87	3.93	2.12	1.26	0.87	3.93

Table 32. Interactive effect of CO₂ and moisture stress on phloem characters of shoot

Treatments	Pusa Gold				RH-30			
	Number	Length (μm)	Breadth (μm)	Area (μm^2)	Number	Length (μm)	Breadth (μm)	Area (μm^2)
FACE irrigated	9.00	5.400	7.000	37.80	14.00	7.900	8.60	67.940
FACE moisture stress	7.00	5.200	6.800	35.36	12.00	7.800	8.50	66.30
Ambient irrigated	7.00	7.10	8.900	63.19	12.00	9.500	10.20	96.90
Ambient moisture stress	5.00	5.0	6.40	32.0	9.00	7.40	8.40	62.16
CV.	0.62	0.52	0.49	2.62	0.62	0.52	0.49	2.62
CO ₂	0.54	0.83	0.83	5.53	0.54	0.83	0.83	5.53
CV. x CO ₂	0.77	NS	NS	8.44	0.77	NS	NS	8.44
MS	0.53	0.51	0.31	1.98	0.53	0.51	0.31	1.98
CV. x MS	0.75	0.93	0.98	2.62	0.75	0.93	0.98	2.62
CO ₂ x MS	0.81	1.53	1.32	3.62	0.81	1.53	1.32	3.62
CV. x CO ₂ x MS	1.06	2.12	2.1	5.95	1.06	2.12	2.1	5.95

whereas, it was 25% (phloem number), 30% (length), 37% (breadth), 37% (area) and 14% (number), 17% (length), 16% (breadth) and 31% (area), respectively in 'RH-30'.

Shoot xylem characters:

Metaxylem

The elevated CO₂ significantly increased the metaxylem number (36%), length (34%), breadth (34%) and area (74%) (Table 33). These parameters were superior in 'RH-30'. Moisture stress significantly reduced the metaxylem number (42%), length (38%), breadth (38%), and area (84%). The stress induced reduction under ambient and elevated CO₂ conditions 33% in number, 31% in length, 31% in breadth and 53% in area and 25% in number, 16% in length, 19% in breadth and 35% in area, respectively in 'Pusa Gold', whereas, it was 30% in number, 31% in length, 31% in breadth and 53% in area and 18% in number, 16% in length, 14% in breadth and 28% in area, respectively in 'RH-30'.

Shoot protoxylem:

Significant increase in proto xylem number (30%), length (35%), breadth (30%) and area (72%) was observed under elevated CO₂ (Table 34). These proto xylem characters were better in 'RH-30'. Moisture stress significantly reduced the proto xylem number (42%), length (41%), breadth (34%), and area (87%). This reduction for xylem characters under ambient and elevated CO₂ conditions was 36% for number, 33% for length, 33% for breadth and 55% for area and 23% for number, 20% for length, 17% for breadth and 34% for area, respectively in 'Pusa Gold', whereas, it was 33% for number, 25% for length, 25% for breadth and 44% for area and 16% for number, 16% for length, 13% for breadth and 27% for area, respectively in 'RH-30'.

Root anatomy:

Vessel characters

CO₂ enrichment brought about significant increase in vessel number (29%),

Table 33. Interactive effect of CO₂ and moisture stress on meta-xylem characters of shoot

Treatments	Pusa Gold					RH-30			
	Number	Length (μm)	Breadth (μm)	Area (μm^2)	Number	Length (μm)	Breadth (μm)	Area (μm^2)	
FACE irrigated	12.00	11.11	13.14	145.86	16.00	14.20	15.10	214.42	
FACE moisture stress	9.00	8.90	10.60	94.34	13.00	11.91	12.90	153.64	
Ambient moisture stress	9.00	9.10	10.90	99.19	13.00	11.97	13.01	155.72	
Ambient moisture stress	6.00	5.90	6.70	39.53	9.00	8.15	8.90	72.54	
CV.	0.62	0.62	0.54	2.83	0.62	0.62	0.54	2.83	
CO ₂	0.31	0.54	0.83	4.98	0.31	0.54	0.83	4.98	
CV. x CO ₂	0.44	0.78	1.18	6.34	0.44	0.78	1.18	6.34	
MS	0.61	0.53	0.31	1.67	0.61	0.53	0.31	1.67	
CV. x MS	0.87	0.75	0.43	4.32	0.87	0.75	0.43	4.32	
CO ₂ x MS	0.92	0.81	0.52	5.02	0.92	0.81	0.52	5.02	
CV. x CO ₂ x MS	1.22	1.06	1.02	6.11	1.22	1.06	1.02	6.11	

Table 34 Interactive effect of elevated CO₂ and moisture stress on protoxylem characters of shoot

Treatments	Pusa Gold					RH-30		
	Number	Length (μm)	Breadth (μm)	Area (μm^2)	Number	Length (μm)	Breadth (μm)	Area (μm^2)
FACE irrigated	13.00	9.10	10.20	92.81	18.00	11.80	12.000	141.60
FACE moisture stress	10.00	7.20	8.40	60.48	15.00	9.90	10.40	102.96
Ambient irrigated	11.00	7.70	8.70	66.99	15.00	10.01	10.70	107.11
Ambient moisture stress	7.00	5.10	5.80	29.58	10.00	7.50	7.93	59.25
CV. CO ₂	0.59	0.47	0.35	7.44	0.59	0.47	0.35	7.44
	1.01	0.69	0.49	10.17	1.01	0.69	0.49	10.17
CV. x CO ₂	1.87	0.89	0.71	12.44	1.87	0.89	0.71	12.44
MS	0.61	0.34	0.42	3.22	0.61	0.34	0.42	3.22
CV. x MS	1.27	0.72	0.73	8.34	1.27	0.72	0.73	8.34
CO ₂ x MS	1.98	0.94	1.02	12.32	1.98	0.94	1.02	12.32
CV. x CO ₂ x MS	2.11	1.67	1.97	16.76	2.11	1.67	1.97	16.76

length (34%), breadth (33%) and area (74%) (Table 35). The root vessel characters were more strategically adjusted in 'RH-30'. Moisture stress adversely affected the vessel characters by reducing their number (38%), length (39%), breadth (37%), and area (82%). The stress-induced reduction in 'Pusa Gold' under ambient and elevated CO₂ condition was 35% in number, 35% in length, 37% in breadth and 59% in area and 21% in number, 17% in length, 16% in breadth and 31% in area, respectively, whereas, it was 30% for number, 33% for length, 33% for breadth and 55% for area and 16% for number, 17% for length, 14% breadth and 28% for area in 'RH-30', respectively.

Root phloem characters

There was significant improvement in phloem characters of roots due to elevated level of CO₂ through increasing in their number (31%), length (32%), breadth (31%) and area (73%) (Table 36). These anatomical characters of phloem were better oriented in 'RH-30' compared to 'Pusa Gold'. Moisture stress treatment adversely affected phloem characters through significant reduction in their number (31%), length (40%), breadth (37%) and area (93%). The stress induced reduction in 'Pusa Gold' under ambient and elevated CO₂ condition was 28% number, 33% (length), 30% (breadth) and 53% (area) and 23% (number), 23% (length), 22% (breadth) and 44% (area), respectively, whereas, it was 25% (number), 25% (length), 27% (breadth) and 46% (area) and 16% (number), 18% (length), 19% (breadth) and 34% (area), respectively in 'RH-30'.

Root xylem characters:

Metaxylem

CO₂ enrichment significantly increased number (31%), length (35%), breadth (32%) and area (80%) of metaxylem in roots. Improved metaxylem characters in roots of 'RH-30' were demonstrated irrespective of treatments (Table 37). Moisture stress reduced the number (38%), length (40%), breadth (41%), and area (92%) of metaxylem in roots. The stress-induced reduction under ambient and elevated

Table 35. Interactive effect of elevated CO₂ and moisture stress on vessel characters of root

Treatments	Pusa Gold					RH-30				
	Number	Length (μm)	Breadth (μm)	Area (μm^2)	Number	Length (μm)	Breadth (μm)	Area (μm^2)		
FACE irrigated	23.00	19.30	25.40	490.22	31.00	25.20	30.20	758.02		
FACE moisture stress	18.00	15.90	21.20	335.49	26.00	20.87	25.90	540.53		
Ambient irrigated	20.00	16.20	21.700	351.54	25.00	20.93	25.90	542.080		
Ambient moisture stress	13.00	10.40	13.600	141.44	17.33	13.90	17.30	240.05		
CV.	0.948	0.56	1.86	2.23	0.948	0.56	1.86	2.23		
CO ₂	1.01	0.63	1.29	3.14	1.01	0.63	1.29	3.14		
CV. x CO ₂	1.43	0.90	1.83	4.02	1.43	0.90	1.83	4.02		
MS	1.63	0.43	1.50	6.12	1.63	0.43	1.50	6.12		
CV. x MS	2.30	1.29	2.54	8.65	2.30	1.29	2.54	8.65		
CO ₂ x MS	2.30	1.32	2.74	8.98	2.30	1.32	2.74	8.98		
CV x CO ₂ x MS	3.26	1.82	4.01	12.24	3.26	1.82	4.01	12.24		

Table 36. Interactive effect of elevated CO₂ and moisture stress on phloem characters of root

Treatments	Pusa Gold				RH-30			
	Number	Length (μm)	Breadth (μm)	Area (μm^2)	Number	Length (μm)	Breadth (μm)	Area (μm^2)
FACE irrigated	17.00	4.20	5.80	24.36	6.40	7.70	7.90	50.56
FACE moisture stress	13.00	4.00	5.60	22.40	6.30	6.30	7.80	49.14
Ambient irrigated	14.00	5.10	7.20	36.72	7.70	6.40	9.80	75.46
Ambient moisture stress	9.00	3.80	5.40	20.52	6.10	4.70	7.60	46.36
CV.	1.86	1.41	1.11	20.11	6.400	0.97	0.34	1.89
CO ₂	1.13	0.30	0.43	2.22	1.13	0.30	0.43	2.22
CV. x CO ₂	1.60	NS	0.80	4.11	1.60	NS	0.80	4.11
MS	1.40	0.21	0.43	5.23	1.40	0.21	0.43	5.23
CV. x MS	1.98	0.31	0.72	6.98	1.98	0.31	0.72	6.98
CO ₂ x MS	2.21	0.62	0.89	7.12	2.21	0.62	0.89	7.12
CV.x CO ₂ x MS	2.98	1.02	1.21	10.42	2.98	1.02	1.21	10.42

Table 37. Interactive effect of elevated CO₂ and moisture stress on metaxylem characters of root

Treatments	Pusa Gold				RH-30			
	Number	Length (μm)	Breadth (μm)	Area (μm^2)	Number	Length (μm)	Breadth (μm)	Area (μm^2)
FACE irrigated	20.00	14.10	17.20	242.52	27.00	18.20	21.90	398.60
FACE moisture stress	16.00	11.40	13.90	158.50	22.00	14.90	18.30	272.67
Ambient irrigated	17.00	11.70	14.20	166.14	22.00	15.01	18.60	279.19
Ambient moisture stress	11.00	7.60	8.70	66.14	15.00	9.97	12.40	123.63
CV.	1.24	0.97	1.24	11.18	1.24	0.97	1.24	11.18
CO ₂	2.67	1.33	1.99	15.21	2.67	1.33	1.99	15.21
CV. X CO ₂	3.77	1.89	2.12	21.51	3.77	1.89	2.12	21.51
MS	1.25	1.02	1.01	10.11	1.25	1.02	1.01	10.11
CV. x MS	2.23	1.98	1.78	12.29	2.23	1.98	1.78	12.29
CO ₂ x MS	3.18	2.12	2.91	14.29	3.18	2.12	2.91	14.29
CV x CO ₂ x MS	4.50	3.04	3.21	20.22	4.50	3.04	3.21	20.22

CO₂ condition was 35% (number), 35% (length), 38% (breadth) and 80% (area) and 20% (number), 19% (length), 19% (breadth) and 34% (area), respectively in 'Pusa Gold', whereas, it was 31% (number), 33% (length), 33% (breadth) and 55% (area) and 18% (number), 18% (length), 16% (breadth) and 31% (area), respectively in 'RH-30'.

Root protoxylem:

CO₂ enrichment increased the number (32%), length (38%), breadth (35%) and area (42%) of root proto xylem (Table 38). Protoxylem number, length, breadth, and area were greater in the roots of 'RH-30'. Moisture stress reduced the protoxylem characters such as their number (42%), length (43%), breadth (39%), and area (44%). The reduction under ambient and elevated CO₂ condition was 40% (number), 35% (length), 34% (breadth) and 57% (area) and 22% (number), 23% (length), 22% (breadth) and 40% (area), respectively in 'Pusa Gold', whereas, it was 35% (number), 31% (length), 30% (breadth) and 51% (area) and 16% (number), 21% (length), 18% (breadth), 36% (area), respectively in 'RH-30'.

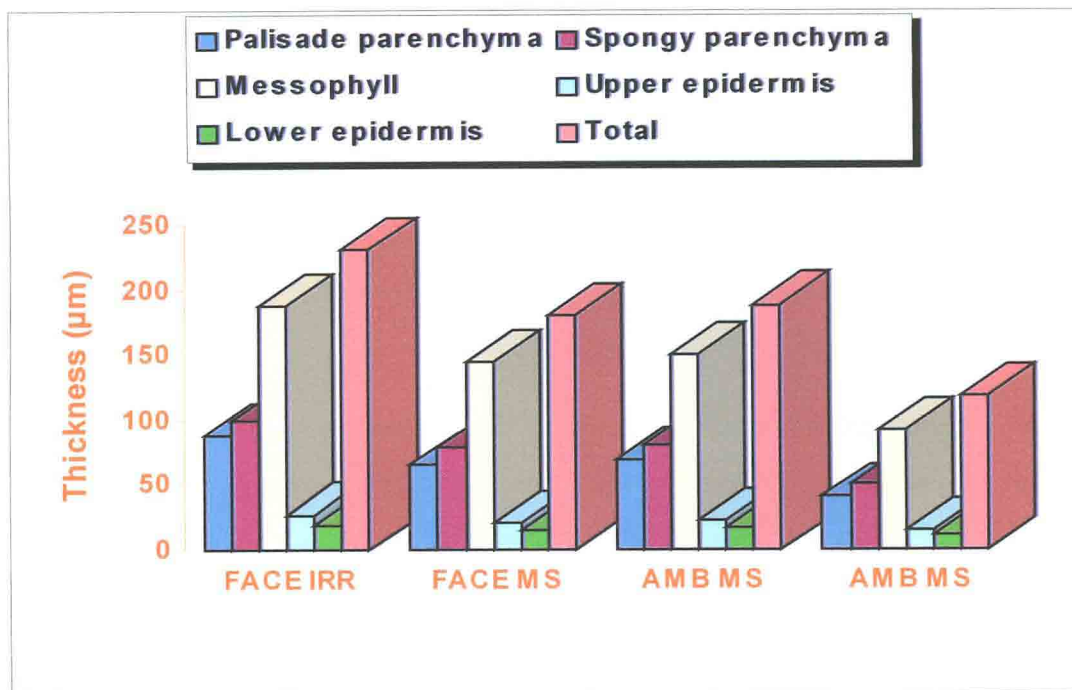
Leaf anatomy:

Leaf thickness

Elevated CO₂ brought about significant increase in the leaf thickness of leaf palisade (39%), spongy (34%), mesophyll (36%), upper epidermis (29%), lower epidermis (28%) and total leaf thickness (34%) (Fig. 41). The leaf thicknesses were significantly greater in 'RH-30' compared to 'Pusa Gold'. Moisture stress adversely affected the leaf thickness by reducing the palisade (22%), spongy (19%), mesophyll (18%), upper epidermis (16%), lower epidermis (25%) and total leaf thickness (18%). The stress induced reduction in 'Pusa Gold' under ambient CO₂ condition was 39% (palisade), 37% (spongy), 38% (mesophyll), 31% (upper epidermis), 35% (lower epidermis), 37% (total thickness), whereas, under elevated CO₂ condition the reduction was 24% for palisade, 21% for spongy, 23% for mesophyll, 22% for upper epidermis, 21% for lower epidermis, 22% for total leaf thickness. The

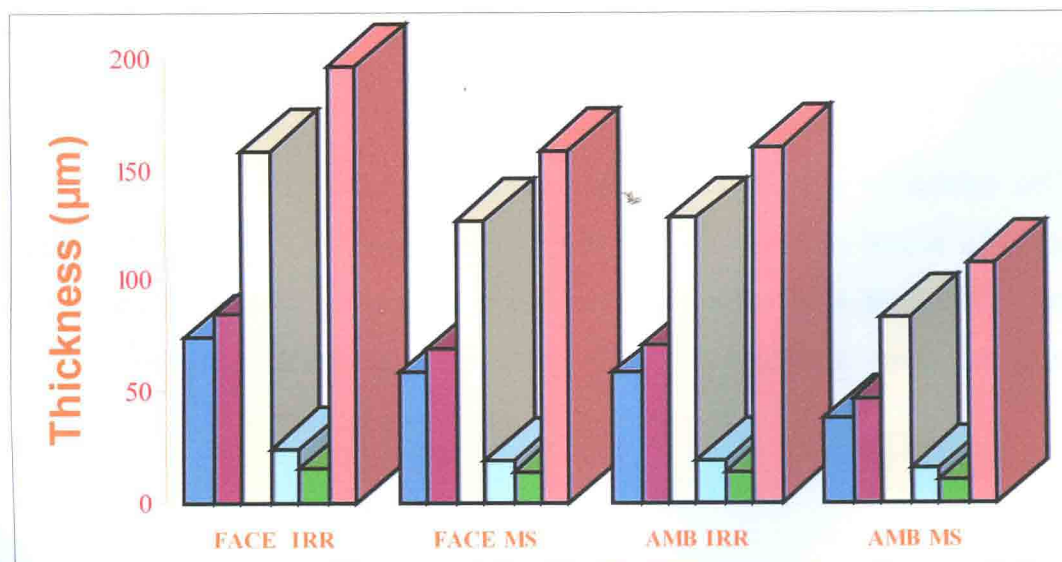
Table 38. Interactive effect of elevated CO₂ and moisture stress on protoxylem characters of root

Treatments	Pusa Gold				RH-30			
	Number	Length (μm)	Breadth (μm)	Area (μm^2)	Number	Length (μm)	Breadth (μm)	Area (μm^2)
FACE irrigated	18.00	16.21	18.40	298.27	24.00	19.10	23.12	441.59
FACE moisture stress	14.00	12.40	14.20	176.09	20.00	14.90	18.90	281.610
Ambient irrigated	15.00	12.70	14.70	186.69	20.00	15.020	19.150	287.63
Ambient moisture stress	9.00	8.21	9.70	79.64	13.00	10.32	13.40	138.28
CV.	0.62	1.86	0.62	5.99	0.62	1.86	0.62	5.99
CO ₂	1.69	2.96	2.01	8.72	1.69	2.96	2.01	8.72
CV. x CO ₂	2.39	4.20	2.85	12.33	2.39	4.20	2.85	12.33
MS	1.64	1.53	1.10	3.67	1.64	1.53	1.10	3.67
CV. x MS	2.33	2.16	1.56	17.35	2.33	2.16	1.56	17.35
CO ₂ x MS	2.67	2.99	1.67	19.45	2.67	2.99	1.67	19.45
CV. x CO ₂ x MS	3.30	3.06	2.21	24.55	3.30	3.06	2.21	24.55



Pusa Gold

Thickness (μm)



RH-30

Fig. 41. Interactive effect of CO₂ and moisture stress on leaf thickness of *Brassica* cultivars

reduction under ambient in 'RH-30' was 35% (palisade), 33% (spongy), 34% (mesophyll), 25% (upper epidermis), 25% (lower epidermis) and 35% (total leaf thickness) and under elevated CO₂ condition the reduction was 21% for palisade, 17% for spongy, 19% for mesophyll, 17% for upper epidermis, 16% for lower epidermis and 19% for total leaf thickness.

Leaf cell size:

Palisade

The increased concentration of CO₂ significantly enhanced the palisade cell length (36%), breadth (23%) and area (65%) (Table 39). The larger palisade cell was observed in leaves of 'RH-30'. Moisture stress reduced its length (38%), breadth (28%) and area (74%). The stress-induced reduction in 'Pusa Gold' under ambient and elevated CO₂ condition was 35% (length), 28% (breadth), and 53% (area) and 22% (length), 16% (breadth), and 35% (area), respectively. Reduction was 28% length, 23% breadth, and 53% (area) and 19% (length), 12% (breadth) and 30% (area), respectively in 'RH-30'.

Spongy

CO₂ enrichment did not significantly affect the size of spongy cell size in leaves (Table 40). The larger spongy cell was observed in leaves of 'Pusa Gold' compared to 'RH-30'. Moisture stress adversely affected it by reducing its length (12%), breadth (12%) and area (21%). CO₂ and moisture stress interaction was not significant for these characters.

Cell number:

Palisade

The increased concentration of CO₂ significantly enhanced (12%) the palisade cell number in *Brassica* cultivars (Table 39). The more number of palisade cells were recorded in leaves of 'RH-30'. Moisture stress treatment significantly reduced (31%) the number of palisade cell. The reduction under ambient and

Table 39. Interactive effect of elevated CO₂ and moisture stress in leaf palisade cell number and cell size

Treatments	Pusa Gold				RH-30			
	Number	Length (μm)	Breadth (μm)	Area (μm^2)	Number	Length (μm)	Breadth (μm)	Area (μm^2)
FACE irrigated	23.00	37.40	22.60	845.24	27.00	48.50	25.90	1256.15
FACE moisture stress	18.00	28.90	18.81	543.61	22.30	38.90	22.60	879.14
Ambient irrigated	19.00	29.20	19.70	575.24	21.00	39.12	22.80	891.94
Ambient moisture stress	13.00	18.90	14.10	266.49	15.90	28.0	17.40	487.20
CV.	1.01	1.67	1.93	37.96	1.01	1.67	1.93	37.96
CO ₂	1.58	2.22	2.45	81.35	1.58	2.22	2.45	81.35
CV. x CO ₂	2.56	3.78	3.47	115.04	2.56	3.78	3.47	115.04
MS	1.67	2.92	2.43	76.90	1.67	2.92	2.43	76.90
CV. x MS	2.87	4.01	3.44	108.75	2.87	4.01	3.44	108.75
CO ₂ x MS	3.54	5.85	3.69	118.75	3.54	5.85	3.69	118.75
CV. x CO ₂ x MS	4.09	7.44	4.86	153.80	4.09	7.44	4.86	153.80

elevated CO₂ was 31% and 21%, respectively in Pusa Gold and 24% and 17%, respectively in 'RH-30'.

Spongy cell

There was no significant effect of CO₂ enrichment on spongy cell number (Table 40). The number of spongy cells were more in leaves of 'Pusa Gold'. Moisture stress treatment significantly reduced (14%) number of spongy cell. CO₂ and moisture stress interaction was not significant.

Stomatal characters in leaves:

Stomatal density

The elevated CO₂ brought about significant reduction in the stomatal density at adaxial and abaxial side of *Brassica* leaves (Table 41). The reduction was 22% (adaxial side) and 25% (abaxial side). The stomatal density was lower in 'RH-30' compared to Pusa Gold. Moisture stress reduced the stomatal density 28% at adaxial side) and 27% at abaxial side. The stress-induced reduction in 'Pusa Gold' under ambient and elevated conditions was 15% (adaxial side) and 19% (abaxial side) and 27% (adaxial side) and 28% (abaxial side), respectively, whereas, reduction was 13% (adaxial side) and 15% (abaxial side) and 23% (adaxial side) and 27% (abaxial side), respectively in 'RH-30'.

Stomatal index

Elevated CO₂ significantly increased the stomatal index at adaxial (25%) and abaxial (28%) side of leaves (Table 41). The higher stomatal index was observed in 'RH-30'. Moisture stress brought about reduction in stomatal index at the adaxial side (30%) and abaxial side (35%) of the leaves. This reduction in 'Pusa Gold' under ambient and elevated CO₂ condition was 26% (adaxial side) and 28% (abaxial side) and 20% (adaxial side) and 22% (abaxial side), respectively. The stress-induced reduction in 'RH-30' under ambient and elevated CO₂ was 22% (adaxial side) and 23% (abaxial side) 16% (adaxial side) and 18% (abaxial side), respectively.

Table 40. Interactive effect of elevated CO₂ and moisture stress leaf spongy cell number and cell size

Treatments	Pusa Gold				RH-30			
	Number	Length (µm)	Breadth (µm)	Area (µm ²)	Number	Length (µm)	Breadth (µm)	Area (µm ²)
FACE irrigated	17.00	31.200	22.900	714.480	14.00	26.600	19.440	517.100
FACE moisture stress	16.00	30.640	21.420	650.180	13.00	24.970	18.910	472.180
Ambient irrigate	18.00	32.620	24.300	792.670	15.00	28.220	22.460	533.640
Ambient moistur stress	15.00	28.620	20.300	580.980	13.00	23.420	18.440	431.860
CV.	1.5	1.56	0.62	50.44	1.5	1.56	0.62	50.44
CO ₂	NS	NS	0.87	NS	NS	NS	0.87	NS
CV. x CO ₂	0.22	NS	1.28	NS	0.22	NS	1.28	NS
MS	0.44	0.83	0.61	18.55	0.44	0.83	0.61	18.55
CV. x MS	0.63	1.13	1.02	30.87	0.63	1.13	1.02	30.87
CO ₂ x MS	0.83	2.44	1.99	42.43	0.83	2.44	1.99	42.43
CV. x CO ₂ x MS	1.21	3.10	2.84	67.54	1.21	3.10	2.84	67.54

Leaf stomatal size:

Adaxial stomata size

The increased concentration of CO₂ significantly increased length (28%), breadth (27%) and area (61%) of stomata at adaxial side (Table 42). The large size of stomata in adaxial side observed in the leaves of 'RH-30'. Moisture stress significantly reduced the stomata size of adaxial side. The reduction was 31% in length, 32% in breadth and 61% in area. The stress-induced reduction in 'Pusa Gold' under ambient and elevated CO₂ was 29% in length, 29% in breadth and 50% in area and 16% in length, 16% in breadth and 30% in area, respectively, whereas, it was 22% in length, 23% in breadth and 39% in area and 14% in length, 15% in breadth and 27% in area in 'RH-30', respectively.

Abaxial stomata size

The higher level of CO₂ increased length (24%), breadth (27%) and area (70%) of the stomata at abaxial side (Table 43). It was larger in 'RH-30'. Moisture stress significantly reduced the stomatal length (33%), breadth (35%) and area (77%) in abaxial side. The stress-induced reduction in 'Pusa Gold' under ambient and elevated CO₂ condition was 30% (length), 31% (breadth), and 52% (area) and 19% (length), 18% (breadth) and 34% (and area), respectively, whereas, it was 26% for length, 29% for breadth, and 47% for area, 17% for length, and 15% for breadth and 30% for area in 'RH-30', respectively.

Stomatal pore length

A significant increase in adaxial (26%) and abaxial (23%) stomatal pore length in *Brassica* was observed with increased concentration of CO₂ (Table 44). The large pore length of stomata was observed for both adaxial and abaxial side of leaves of 'RH-30' compared to 'Pusa Gold'. Moisture stress significantly reduced stomatal pore length from adaxial side (32%) and abaxial side (34%). The stress-induced reduction in 'Pusa Gold' under ambient and elevated CO₂ conditions was 30% (adaxial side) and 31% (abaxial side) and 18%, respectively, whereas, it was

Table 42 Interactive effect of elevated CO₂ and moisture stress on leaf adaxial stomatal size

Treatments	Pusa Gold			RH-30		
	Length (μm)	Breadth (μm)	Area (μm^2)	Length (μm)	Breadth (μm)	Area (μm^2)
FACE irrigated	15.20	12.40	188.480	18.10	16.40	296.84
FACE moisture stress	12.70	10.30	130.810	15.50	13.90	215.45
Ambient irrigated	12.90	10.60	136.74	15.70	14.10	221.37
Ambient moisture stress	9.100	7.50	68.250	12.20	10.90	132.98
CV, CO ₂	0.62	0.58	6.93	0.62	0.58	6.93
	0.44	0.39	8.77	0.44	0.39	8.77
CV. x CO ₂	0.63	0.57	9.98	0.63	0.57	9.98
MS	0.53	0.49	4.49	0.53	0.49	4.49
CV. x MS	0.74	0.70	8.91	0.74	0.70	8.91
CO ₂ x MS	0.85	0.81	10.47	0.85	0.81	10.47
CV. x CO ₂ x MS	1.43	1.37	14.72	1.43	1.37	14.72

Table 43. Interactive effect of elevated CO₂ and moisture stress leaf stomatal size

Treatments	Abaxial					
	Pusa Gold			RH-30		
	Length (μm)	Breadth (μm)	Area (μm^2)	Length (μm)	Breadth (μm)	Area (μm^2)
FACE irrigated	16.70	13.70	228.79	19.60	17.70	346.92
FACE moisture stress	13.40	11.20	150.08	16.20	14.90	241.92
Ambient irrigated	14.60	11.90	173.74	16.90	15.40	260.26
Ambient moisture stress	10.10	8.20	82.82	12.40	10.90	135.58
CV.	1.86	1.43	4.97	1.86	1.43	4.97
CO ₂	0.70	0.61	7.67	0.70	0.61	7.67
CV. x CO ₂	0.99	0.93	8.84	0.99	0.93	8.84
MS	1.01	0.56	4.32	1.01	0.56	4.32
CV. x MS	1.43	1.11	7.98	1.43	1.11	7.98
CO ₂ x MS	1.73	1.54	9.97	1.73	1.54	9.97
CV. x CO ₂ x MS	2.02	1.89	13.44	2.02	1.89	13.44

Table 44. Interactive effect of elevated CO₂ and moisture stress on stomatal pore length.

Treatments	Leaf adaxial				Leaf abaxial				Stem		Siliquae	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	8.80	11.10	10.10	13.30	4.60	6.30	4.20	6.10				
FACE moisture stress	7.20	9.40	8.20	11.70	3.90	5.50	3.60	5.39				
Ambient irrigated	7.50	9.90	9.30	11.10	4.00	5.20	3.80	5.40				
Ambient moisture stress	5.20	7.40	6.40	8.50	3.10	4.10	2.90	4.10				
CV.	0.67			0.62		0.23		0.19				
CO ₂	0.78			0.83		0.44		0.35				
CV. x CO ₂	1.01			1.17		0.62		0.58				
MS	0.33			0.31		0.43		0.31				
CV. x MS	0.88			0.43		0.61		0.52				
CO ₂ x MS	0.93			0.97		0.71		0.69				
CV. x CO ₂ x MS	1.67			1.56		0.86		0.81				

25% (adaxial) and 23% (abaxial) and 15% (adaxial) and 12% (abaxial), respectively in 'RH-30'.

Stem stomatal characters:

Stomatal density

The increased concentration of CO₂ brought about significant reduction (28%.) in the stomatal density in *Brassica* stem (Table 45). The stomatal density was lower in 'RH-30' compared to 'Pusa Gold' in the stem. Moisture stress treatment significantly reduced (37%) the stomatal density in *Brassica* stem. The stress-induced reduction in 'Pusa Gold' under ambient and elevated CO₂ condition was 20% and 20%, respectively, whereas, 33% and 20%, respectively in 'RH-30'.

Stomatal index

The elevated CO₂ significantly increase in the stomatal index *Brassica* stem (31%) (Table 45). The stomatal index was higher in 'RH-30' compared to 'Pusa Gold'. Moisture stress treatment significantly reduced the (35%) stomatal index of *Brassica* stems. The stress-induced reduction in 'Pusa Gold' under ambient and elevated CO₂ was 32% and 17%, respectively, whereas this reduction in 'RH-30' was 32% and 14%, respectively.

Stomata size

Elevated CO₂ significantly increased the length (36%), breadth (34%) and area (79%) of stomata (Table 46). The large size of stomata was observed in the stem of 'RH-30' compared to 'Pusa Gold'. Moisture stress reduced the length (41%), breadth (40%) and area (93%) of stomata. The stress-induced reduction in 'Pusa Gold' under ambient and elevated CO₂ condition was 35% for length, 35% for breadth, and 58% for area and 24% for length, 21% for breadth and 40% for area, respectively in 'Pusa Gold', whereas, it was 28% for length, 30% for breadth, and 50% for area and 16% for length, 15% for breadth and 29% for area in 'RH-30', respectively.

Table 45. Interactive effect of elevated CO₂ and moisture stress stem and silique stomatal density and intensity

Treatments	Stem						Silique					
	Pusa Gold			RH- 30			Pusa Gold			RH- 30		
	Density	Intensity	Density	Intensity	Density	Intensity	Density	Intensity	Density	Intensity	Density	Intensity
FACE irrigated	86.70	8.40	43.20	10.80	74.40	7.80	50.10	9.80				
FACE moisture stress	69.20	6.90	34.10	9.20	59.10	6.30	37.30	8.30				
Ambient irrigated	123.30	7.10	57.400	9.60	97.30	6.50	72.20	8.50				
Ambient moisture stress	82.40	4.80	38.20	6.90	68.10	4.30	48.10	6.20				
CV.	1.97	0.43	1.97	0.43	1.86	0.39	1.86	0.39				
CO ₂	4.38	0.61	4.38	0.61	1.57	0.74	1.57	0.74				
CV. x CO ₂	6.19	0.86	6.19	0.86	2.22	0.82	2.22	0.82				
MS	2.53	0.39	2.53	0.39	3.71	0.51	3.71	0.51				
CV. x MS	5.08	0.63	5.08	0.63	5.25	0.66	5.25	0.66				
CO ₂ x MS	6.14	0.90	6.14	0.90	6.01	0.86	6.01	0.86				
CV. x CO ₂ x MS	8.34	1.21	8.34	1.21	7.42	1.32	7.42	0.96				

Table 46 Interactive effect of elevated CO₂ and moisture stress stem stomatal size

Treatment	Stem						Siliquae					
	Pusa Gold			RH-30			Pusa Gold			RH-30		
	Length (μm)	Breadth (μm)	Area (μm^2)	Length (μm)	Breadth (μm)	Area (μm^2)	Length (μm)	Breadth (μm)	Area (μm^2)	Length (μm)	Breadth (μm)	Area (μm^2)
FACE irrigated	10.40	9.70	100.80	12.80	12.10	154.88	9.90	9.30	92.07	12.20	11.90	145.11
FACE moisture stress	7.90	7.60	60.04	10.7	10.20	109.14	7.70	7.40	56.98	10.10	9.90	99.90
Ambient irrigated	8.10	7.90	63.99	10.80	10.50	113.40	7.80	7.60	59.28	10.40	10.10	105.01
Ambient moisture stress	5.20	5.10	26.52	7.70	7.30	56.21	4.90	4.70	23.03	7.20	7.200	51.84
CV.	1.67	1.47	7.63	1.67	1.47	7.63	0.48	0.58	4.95	0.48	0.58	4.95
CO₂	0.44	0.56	3.25	0.44	0.56	3.25	0.67	0.73	5.72	0.67	0.73	5.72
CV. x CO₂	0.63	0.89	4.60	0.63	0.89	4.60	0.97	1.03	6.89	0.97	1.03	6.89
MS	0.75	0.65	5.27	0.75	0.65	5.27	0.44	0.42	3.50	0.44	0.42	3.50
CV. x MS	1.05	1.12	7.46	1.05	1.12	7.46	0.62	0.69	5.21	0.62	0.69	5.21
CO₂ x MS	1.56	1.47	8.19	1.56	1.47	8.19	0.86	0.92	7.35	0.86	0.92	7.35
CV. x CO₂ x MS	1.98	1.89	10.54	1.98	1.89	10.54	1.26	1.34	11.24	1.26	1.34	11.24

Stomatal pore length

Pore length of stem stomata was increased by 28% due to elevated CO₂. It was larger in 'RH-30' (Table 44). Moisture stress treatment significantly reduced (30%) the stomatal pore length of stem. This reduction under ambient and elevated CO₂ condition was 23% and 15%, respectively in 'Pusa Gold' and 21% and 12%, respectively in 'RH-30'.

Siliquae stomatal parameter:

Stomatal density

The elevated level of CO₂ brought about significant reduction in stomatal density (32%) of siliquae (Table 45). The stomatal density was lower in 'RH-30'. Moisture stress treatment significantly reduced (37%) the stomatal density of siliquae of *Brassica*. The stress-induced reduction in 'Pusa Gold' under ambient and elevated CO₂ conditions was 19% and 21%, respectively and 15% and 25% in 'RH-30', respectively.

Stomatal index

The increased concentration of CO₂ significantly increased (32%) the stomatal index in siliquae of *Brassica* (Table 45). The stomatal index was higher in the siliquae of 'RH-30'. Moisture stress reduced (35%) the stomatal index. The stress-induced reduction in 'Pusa Gold' under ambient and elevated CO₂ was 33% and 19%, respectively, whereas, it was 27% and 15%, respectively in 'RH-30'.

Siliquae stomata size

The elevated CO₂ significantly increased the length (23%), breadth (24%) and area (54%) of siliquae stomata (Table 46). The large size of stomata was recorded in the siliquae of 'RH-30' compared to 'Pusa Gold'. Moisture stress significantly reduced its length (40%), breadth (41%) and area (92%). This reduction in 'Pusa Gold' under ambient and elevated condition was 37% for length, 38% for breadth and 61% for area and 22% for length, 20% for breadth and 38%

for area, respectively, similarly, it was 30% for length, 28% for breadth and 50% for area and 17% for length, 16% for breadth and 31% for area, respectively in 'RH-30'.

Stomatal pore length

CO₂ enrichment significantly increased the (27%) length of the pore of siliquae (Table 44). The large size of pore length of siliquae in stomata was observed in 'RH-30'. Moisture stress significantly reduced (32%) the stomatal pore length of siliquae. The stress induced reduction in 'Pusa Gold' under ambient and elevated CO₂ condition was 23% and 14%, respectively, whereas, it was 20 and 12%, respectively in 'RH-30'.

Chloroplast ultra-structure:

Number of chloroplast in mesophyll cell

The CO₂ enrichment brought about significant increase (72%) in the number of chloroplast in the mesophyll cell (Table 47). The more number of chloroplast were recorded in 'RH-30' cultivar compared to 'Pusa Gold'. Moisture stress significantly decreased (25%) the chloroplast number in mesophyll cell. The stress-induced reduction in chloroplast number under ambient and elevated CO₂ conditions was 34% and 22%, respectively in 'Pusa Gold', whereas, it was 32%, and 19%, respectively in 'RH-30'.

Number of chloroplast in vascular sheath cell

The CO₂ enrichment brought about significant increase (36%) in the number of chloroplast in vascular sheath cell (Table 47). There was no significant difference in cultivars for this character. Moisture stress significantly decreased (25%) the chloroplast number in vascular bundle cell. The stress-induced reduction in chloroplast number under ambient and elevated CO₂ condition was 34% and 21%, respectively in 'Pusa Gold' and 33% and 17%, respectively in 'RH-30'.

Table 47. Interactive effect of elevated CO₂ and moisture stress on ultra structure of chloroplast

Treatments	Number of chloroplast in messophyll		Number of starch granule in mesophyll chloroplast		Number of chloroplast in vascular sheath		Number of starch granule in vascular sheath chloroplast	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	7.50	8.90	3.70	3.90	3.20	3.50	3.00	3.20
FACE moisture stress	5.80	7.20	2.80	3.10	2.50	2.90	2.40	2.70
Ambient irrigated	4.70	5.50	2.90	2.90	2.60	2.70	1.90	1.80
Ambient moisture stress	3.10	3.70	1.50	1.50	1.70	1.80	1.10	1.20
CV.	0.84			NS		NS		NS
CO ₂	0.11			0.08		0.09		0.07
CV. x CO ₂	0.18			0.12		0.12		0.10
MS	0.09			0.11		0.06		0.05
CV. x MS	0.43			0.22		0.12		0.11
CO ₂ x MS	0.67			0.43		0.30		0.21
CV. x CO ₂ x MS	1.42			0.61		0.42		0.30

Number of starch granule in mesophyll chloroplast

Elevated CO₂ brought about significant increase (35%) in the number of starch granule in the mesophyll chloroplast (Table 47). Moisture stress significantly decreased (33%) the number of starch granule in the mesophyll chloroplast. The reduction was 48% under ambient condition and 24% under elevated CO₂ in 'Pusa Gold' and 46% and 21%, respectively in 'RH-30'.

Number of starch granule in vascular sheath chloroplast

The CO₂ enrichment significantly increased (41%) and moisture stress significantly decreased (19%) the starch granule in the vascular sheath chloroplast (Table 47). This reduction under ambient and elevated CO₂ condition was 42% and 20%, respectively in 'Pusa Gold', whereas, 33% and 15% in 'RH-30', respectively.

5. DISCUSSION

The CO₂ concentration in the atmosphere is rising and is anticipated to be doubled by the end of the next century (Houghton, 1990). This is likely consequence of CO₂ emission from fossil fuel combustion and land use changes. The elevated CO₂ is expected to cause global warming and would also change the carbon balance in the biosphere by affecting the photosynthetic carbon assimilation in plants (Bazzaz, 1990). These changes would affect the agro-ecosystem both climatically and biologically. The agricultural crop production is one of the key sectors that might be affected by the rising atmospheric CO₂ with consequence on the global food security through its effect on photosynthetic rates and thus productivity.

It was reported that C₃ plants (wheat, rice, oilseeds, pulses etc.) respond to elevated CO₂ by reducing the oxygenase activity of RuBP carboxylase oxygenase enzyme, changes in stomatal conductance, root growth and water use efficiency. C₄ plants (Sorghum, maize, sugarcane etc.) show little or feeble response to their photosynthesis because the C₄ pathway is completely CO₂ saturated (Poorter, 1993). However, there is no consensus on the quantitative effects of increased CO₂ on plant processes and growth due to differences in response at different stages of growth, species of crops and because of growth limiting environmental factors.

Innovative approaches for conducting long-term experiments had been developed to investigate the impact of rising atmospheric CO₂ on crops. Such studies are required as our crop varieties were selected for the current CO₂ and their response to the doubling CO₂ concentration are required to be characterized for developing a plant type for future CO₂ changes.

Western studies on the response of plant to high CO₂ concentration were

conducted in closed chambers using short-term exposure in crops of temperate climate. However, studies on tropical/subtropical crops were required to generate data base on the responses of these crops and to characterize those responses morpho-physiologically for tailoring plant species and determining nutrient and cultivation management of those plants for changed climatic condition. These data will help to construct model to predict growth and productivity so that farmers could utilize this information for future.

Free Air CO₂ Enrichment Technology (FACE), which was developed for the first time in this region, was used in the present investigation besides Open Top Chambers (OTC) modified for South Asian region (Upreti *et al.*, 2000 and Maini *et al.*, 2002). This technology has many advantages over other closed chamber systems used by earlier scientist (Upreti *et al.*, 1995, 1998). FACE could enrich a larger area of vegetation with CO₂, without confounding effect of growth chamber, for extended period of time and lower cost per unit area than any other techniques. Both OTCs and FACE provide almost natural condition for the generation of realistic biological data on crop response to the rising atmospheric CO₂ (Upreti *et al.*, 2000 and Kimball *et al.*, 2002).

The crop used in the current study was *Brassica*, an important oil seed of Northwestern India. This crop experiences intermittent drought during its growth period from vegetative to the siliquae formation stage, and there was variability in the responses of these cultivars to the moisture stress (Chopra and Shyam Prakash, 1991 and Upreti *et al.*, 1995). Thus it was considered important to study the interactive effect of the elevated CO₂ and moisture stress, on the morpho-physiological processes of *Brassica* species namely *B. campestris* cv. 'Pusa Gold' and *B. juncea* cv. 'RH-30' in the present investigation.

An extensive description of the experimental findings has been given in 'results' but it will be worthwhile to recapitulate the salient features before discussing them.

The present investigation revealed that the elevated CO_2 significantly increased the rate of photosynthesis and internal CO_2 concentration in *Brassica* spp. throughout the growth period. The highest photosynthetic rate, intercellular CO_2 concentration and decreased level of stomatal conductance was observed at flowering stage indicating the maximum responsiveness of *Brassica* cultivars to the elevated CO_2 . The response was greater in the 'RH-30' cultivar compared to 'Pusa Gold'. The moisture stress treatment brought about significant reduction in the photosynthetic rate and intercellular CO_2 concentration. This adverse effect was lesser under elevated CO_2 .

The chlorophyll contents (Chl a, Chl b and total Chl) of leaves were reduced under higher CO_2 . Chlorophyll content was significantly more in 'RH-30' cultivar throughout the growth period. Moisture stress adversely affected the chlorophyll concentration in leaves. However, reduction in the chlorophyll content was ameliorated by the CO_2 enrichment due to its slow degradation with age.

Elevated CO_2 brought about a significant reduction in the rate of leaf respiration throughout the growth period. The reduction in respiration rate was also observed under moisture stress condition. This adverse effect on respiration was lesser under elevated CO_2 . The photosynthesis: respiration ratio was higher under elevated level of CO_2 at each stage of growth.

Increased rate of photosynthesis and reduction in respiration are two important general effects of elevated CO_2 in crop plants (Acock and Allen, 1985). Photosynthesis and respiration processes were re-examined to characterize the response of *Brassica* spp. to elevated CO_2 at different stages of growth. It was demonstrated that CO_2 enrichment brought about a marked increase in the rate of photosynthesis throughout the growth period in both the cultivars. The increase was substantiated by significantly higher intercellular CO_2 concentration, reduced respiration and stomatal conductance.

Increased rate of photosynthesis was related to CO_2 induced inhibition of oxygenation of Ribulose 1, 5 bis phosphate carboxylase oxygenase enzyme thus,

partially suppressing photorespiration and increasing velocity of carboxylation reaction (Cousins *et al.*, 2001). However, in the present study the enzyme RuBP carboxylase oxygenase was not studied. The response of leaves to an elevated CO₂ concentration possibly depend on the inherent sink strength of plants (Upreti *et al.*, 1998, Makino and Mae, 1999) and this finding was also supported by Morinaga (2002). According to him the sink capacity of fruit may be important for the maintenance of photosynthesis in long-term exposure to an elevated CO₂ concentration. He observed that the non-bearing *Satsuma madarine* trees had a rapid decrease in photosynthesis whereas, it increased in fruit bearing trees. In the present investigation 'RH-30' with large number of leaves and siliquae and greater sink strength responded more to elevated CO₂ for photosynthesis than that of 'Pusa Gold'.

It was also observed that the plants with higher leaf thickness due to elevated CO₂ showed high rate of photosynthesis. The photosynthetic capacity per unit leaf area increased as a result of increasing leaf thickness accompanied by production of an additional layer of palisade cells. The CO₂ enrichment may stimulate the anticlinal divisions of mesophyll cells leading to the production of additional palisade layers to give greater resource utilization area for the additional CO₂ in the mesophylls of *Brassica* leaves. Hofstra and Hesketh (1975) recorded such an increase in leaf thickness in Soyabean.

Present study also demonstrated that chlorophyll content was reduced significantly under elevated CO₂ in *Brassica* leaves. The significant reduction was attributed to the reduction in Rubisco protein by Wilkins *et al.* (1994) and to the inhibition of the transcription of *cab* genes due to accumulation of soluble sugar by Van Oosten *et al.* (1994). In the present investigation, larger accumulation of non-structural carbohydrates appeared to be related to the reduction in the concentration of chlorophyll pigment indicating a dilution effect, which was maintained throughout the growth period due to slow degradation of chlorophyll. Upreti *et al.* (2002) demonstrated that reduction in Rubisco proteins could not

deter the stimulation of photosynthetic efficiency by compensating it through increase in light harvesting complex (LHC) proteins in mature leaves by decreasing the requirement of photon fluxes for Rubisco. Elevated CO₂ induced reduction in chloroplast protein through carbohydrate suppression of gene expression in mature leaves and developing leaves would provide their own sink for carbohydrates.

The lower rate of photosynthesis under moisture stress, which might be due to low internal CO₂, was greatly ameliorated by CO₂ enrichment indicating that stomates were one of the main limiting factors for carbon uptake under moisture stress condition. As in the present study, several other studies (Tyree and Alexander, 1993 and Samarakoon *et al.*, 1995) attributed it partially to the depression in stomatal conductance. Turner (1974) demonstrated that stomatal conductance of plants under high CO₂ concentration was not reduced as rapidly during stress as did under ambient CO₂ level. Sebastiani *et al.* (2002) reported that the elevated CO₂ enhanced rates of net photosynthesis and decreased stomatal conductance in *Olea europaea*, contributing to higher water use efficiency. Drought stress reduced photosynthate production in wheat because of stomatal closure. This might be partially overcome by an increase in air CO₂ concentration (Li *et al.*, 2000).

The decline in dark respiration in *Brassica* leaves at elevated CO₂ was observed in the present investigation. This result is in accordance with the findings of (Amthor, 1997; Drake *et al.*, 1997 and Curtis and Wang, 1998). Wullschleger and Norby (1992) demonstrated a decrease of 12-37 per cent in growth respiration and 45-54 per cent in maintenance respiration in the leaves of white oak sapling grown at elevated CO₂. Ziska and Bunce (1993) attributed this to a larger decrease in maintenance respiration. Although the maintenance respiration is a small fraction of total energy cost, its increase is significant in rapidly growing plant as the plant size increases.

Though, in the present investigation the growth and maintenance respiration were not measured separately, the rapid inhibitory effect of elevated CO₂ on efflux rates could be related to the inhibition of the activity of some respiratory enzymes

e.g. Succinate dehydrogenase (Shaish *et al.*, 1989) or cytochrome c oxidase (Gonzalez-Meler *et al.*, 1996). Bunce (2001) demonstrated that elevated CO₂ inhibited respiration to a greater extent due to non-substrate availability. However, the mechanism such as carboxylation of protein or direct inhibition of respiratory enzyme cannot adequately explain the amount of suppression. Elevated CO₂ may also influence leaf respiration directly by altering growth rate, accumulation of non-structural carbohydrates and other changes in tissue composition (Hamilton *et al.*, 2001). Imai (1995) asserted that the suppressed dark respiration at high CO₂ might generally increase the net production of rice unless it was too low to adversely affect the energetic processes.

Thus, there is variation in maintenance respiration as the dark respiration may significantly alter the total carbon budget. Amthor (1994) argued that with the exception of actively growing cells, respiration was regulated mainly by the rate at which respiratory products ATP, NAD(P)H and the carbon skeleton was used to meet the metabolic demands of growth, maintenance, transport and ion uptake. The higher photosynthetic rates reduce the demand for respiratory energy by making ATP and NAD(P)H more available (Turpin and Wager, 1990). The availability of photochemical energy might be greater at elevated CO₂ because of the suppression of photorespiration and subsequent conservation of photochemical energy, which could be utilized for carbon assimilation. The CO₂ induced reduction of dark respiration may also be explained on the basis of: (a) the reduction in nitrogen and protein content of the tissue (Wullschleger *et al.*, 1994 and Amthor, 1995) (b) the inhibition of some respiratory enzymes as observed in *Scirpus olneyi* and *Lindera benzoin* (Azcon-Bieto *et al.*, 1994) (c) the lesser respiratory requirement as most of biomass was used for structural parts of plants and (d) the use of cyanide insensitive respiration which was lesser as observed in wheat (Gifford *et al.*, 1985).

These confirmed the present findings in *Brassica* where the additional establishment of structural and storage tissue had not been affected by reduced respiration as their respiratory requirement was substantially reduced. The decline

in respiration in *Brassica* under moisture stress was greater at ambient CO₂ than at elevated CO₂, which indicated the compensatory mechanism of carbon balance and energy conservation.

The CO₂ enrichment leads to significant increase in different carbohydrates such as non-reducing, reducing and total sugars in different parts of the plant throughout the growth period. The higher amount of leaf carbohydrate components was observed during the time of flowering. But in case of root and shoot higher amount was observed at pre-flowering stage. All the carbohydrate components were decreased during the beginning of post flowering stage indicating that the mobilization of carbohydrates started from stem and roots to other sinks. Smart *et al.* (1994) observed variation in the distribution of carbohydrates in the leaves due to elevated CO₂. The accumulation of starch, fructans, sucrose and non-structural carbohydrates were in the upper layer of leaves while starch and fructans accumulated in lower canopy layer.

The carbon accumulation in various plant organs such as leaf, stem and roots was significantly higher at elevated level of CO₂. The higher carbon content was observed in the leaf during flowering. But the greater accumulation of carbon in roots and stem was found at pre-flowering stage. The reduction in carbon content during flowering stage may attribute to the carbohydrate components diverted from these organs to the other sinks for reproductive development.

The observations made in the present study indicated an obvious change in the leaf chemical composition with larger accumulation of carbohydrate and concurrent decline in N content. The decline in N content has been attributed to (a) reallocation of N for Rubisco (b) to large carbohydrate accumulation and (c) shift in plant size at higher CO₂.

Present study also reported the accumulation of more carbohydrate in terms of total sugars, starch and non-structural carbohydrates due to CO₂ enrichment. According to Stitt (1991) and Sage (1994), the excessive accumulation of carbohydrates in leaves was accepted to be one of the most important determinants

for the development of new sinks. This excess carbohydrate helped in generation and development of more new sink, which contribute to productivity. Chen (2002) reported that elevated CO₂ concentration increased carbohydrate production of apple trees. Both content and accumulation of non-structural carbohydrates particularly, starch and sucrose, increased in all organs of the trees under the doubled CO₂ condition. It increased non-structural carbohydrate production efficiency of leaves especially starch production efficiency and consequently enhance the fruit production in apple trees.

The C/N ratio of different plant parts was significantly increased under elevated CO₂. The rate of C and N assimilation and turnover determine the C/N ratio of the plant. This ratio generally tends to increase as a result of CO₂ enrichment. The increase in C/N ratio could be explained in terms of (a) dilution of nitrogen due to higher carbohydrate concentration (b) a decreased demand of N in green tissue due to decreased content of Rubisco (c) the depression in photorespiratory pathway resulting in less N demand because of smaller content of enzymes of glycolate pathway and (d) increase in the sink size proportionally under sufficient nutrients.

All these changes were observed in *Brassica* species in the present study as well as investigation made earlier in rice (Upreti *et al.*, 2002). According to Conroy and Hocking (1993) an increased limitation of N assimilates partitioning was responsible for the N deficit. The tendency towards increased C/N ratio by CO₂ enrichment caused N limitation in growth and this must be overcome through nitrogen nutrient management (Riverie-Ronald *et al.*, 1996 and Upreti and Mahalakshmi 2000).

The protein content was significantly reduced in the leaves of *Brassica* cultivars at higher CO₂. Moisture stress has no effect on protein content in the present investigation. This may be due low carbon accumulation under moisture stress. Schwanz *et al.* (1996) attributed less accumulation of carbohydrate under drought due to the reduction in dry weight: fresh weight ratio. In the present study,

protein although lower in quantity, sustained itself for a longer period under elevated CO₂ condition.

Plant height, branch number and stem girth (Plate 1) in *Brassica* spp were significantly increased due to CO₂ enrichment. Moisture stress treatment reduced these growth parameters. The stress induced reduction of growth parameters greatly ameliorated by elevated CO₂. CO₂ enrichment enhanced the leaf number (Plate 2 & 3), leaf area (Plate 4), leaf area index and leaf area duration at each stages of growth. All the above parameters were significantly increased at flowering stage. Moisture stress reduced these parameters significantly. This adverse effect on these parameters was lesser under elevated CO₂ condition.

Present investigation revealed that the elevated CO₂ brought about significant increase in root length, volume, and number of secondary roots (Plate 5) and root: shoot ratio. Moisture stress treatment reduced all these parameters. The CO₂ enrichment ameliorated the stress induced effect on these parameters.

Derner *et al.* (2001) reported that both root and shoot systems of C₄ grass (*Tallgrass prairie*, *Schizachyrium scoparium* and *Andropogon gerardii*) seedlings responded similarly to CO₂ enrichment irrespective of whether soil water was limiting or abundant. The relative distribution of root surface area, number of root tips and length and volume of roots were significantly affected by CO₂ enrichment with proportional increase of 55-61 per cent, 39-52 per cent, 50-55 per cent and 53-58 per cent, respectively.

In the present investigation the CO₂ induced dry-matter accumulation in roots was mainly due to increase in the root length and root numbers, whereas, the shoot growth increased in term of height, branch number as well as diameter, and in leaves, the increase was in terms of their numbers, thickness and surface area (Plate 4).

The high root : shoot ratio under elevated CO₂ was attributed to longer root system with greater biomass partitioning to below ground mass as observed



**FACE
irrigated**

**FACE
moisture stress**

**Ambient
irrigated**

**Ambient
moisture stress**

Pusa Gold



**FACE
irrigated**

**FACE
moisture stress**

**Ambient
irrigated**

**Ambient
moisture stress**

RH-30

**Plate. 1 Interactive effect of elevated CO₂ and moisture stress on
stem girth of *Brassica* cultivars**



FACE irrigated



FACE moisture stress



Ambient irrigated



Ambient moisture stress

Plate. 2 Interactive effect of elevated CO₂ and moisture stress on plant size of *Brassica campestris* cv. Pusa Gold



FACE Irrigated



FACE moisture stress



Ambient irrigated



Ambient moisture stress

**Plate. 3 Interactive effect of elevated CO₂ and moisture stress
on plant size of *Brassica juncea* cv. RH-30**



FACE irrigated



FACE moisture stress



Ambient irrigated



Ambient moisture stress

Pusa Gold



FACE irrigated



Ambient moisture stress



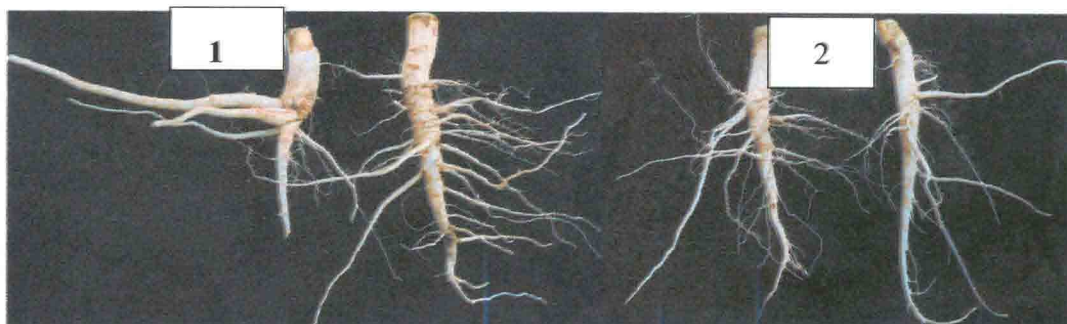
Ambient irrigated



Ambient moisture stress

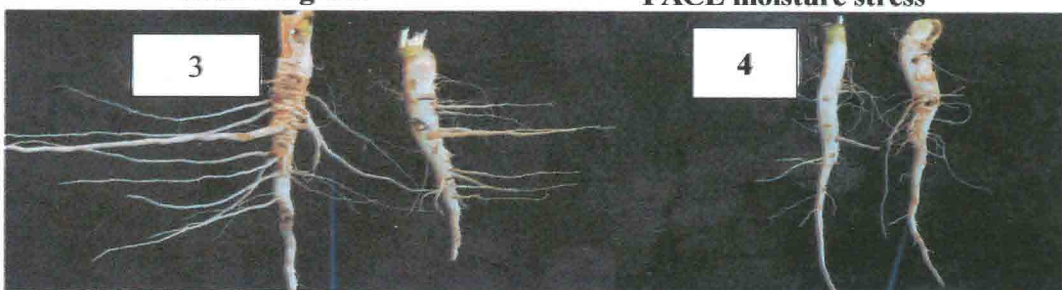
RH-30

Plate.4 Interactive effect of elevated CO₂ and moisture stress on leaf size of *Brassica* cultivars



FACE irrigated

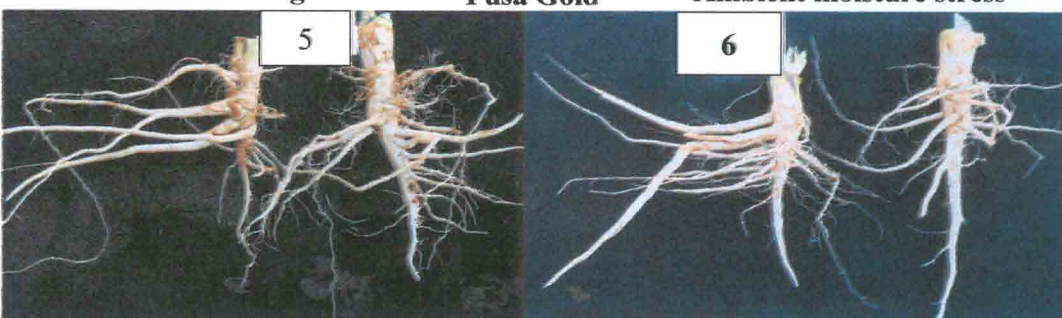
FACE moisture stress



Ambient irrigated

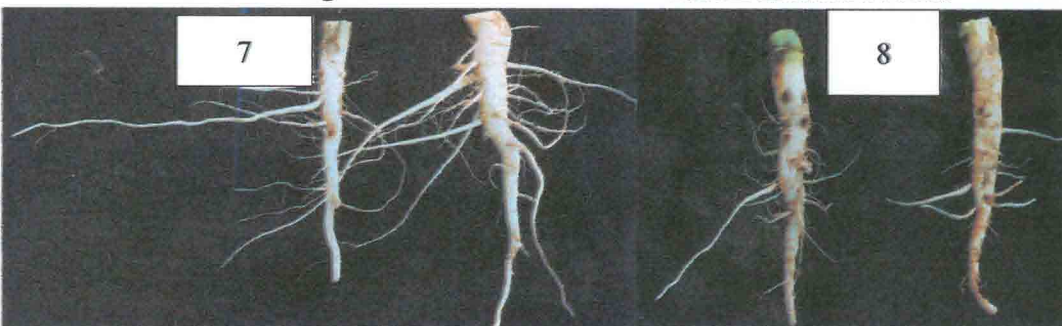
Pusa Gold

Ambient moisture stress



FACE irrigated

FACE moisture stress



FACE irrigated

RH -30

FACE moisture stress

Plate 5 Effect of elevated CO₂ and moisture stress on root size of *Brassica* cultivars

by Vessey *et al.* (1990) and Uprety *et al.* (1995). They reported that the root-derived carbon accounts between 10 and 40 per cent of the total assimilated carbon. Effect of elevated CO_2 on nonstructural carbohydrate partitioning was poorly understood from a mechanistic perspective. Evidence suggested that, in elevated CO_2 levels, there was greater photosynthesis and nonstructural carbohydrate accumulation, which initially partitioned more carbon to roots compared to shoots. The increased root growth under elevated CO_2 conditions permit water extraction from soil, which could be important in a future warmer and drier environment.

Morrison (1983) suggested that greater carbon allocation to roots as a mechanism to improve plant water status at elevated CO_2 . According to Norby *et al.* (1986), the dry mass of tap roots increased 93 per cent and fine roots 110 per cent in *Quercus alba*. The capacity of root system to take up water depends not only root mass but also rooting volume (rooting depth), fine root area and activity. The acceleration of root growth in elevated CO_2 could also result in the establishment of seedlings more rapidly and avoiding water deficit. Polle *et al.* (1996) observed in terrestrial grasses an increase in the ratio of lateral roots to total root mass and lateral root to leaf area. Thus, this increase in root growth at elevated CO_2 has enhanced water uptake, improved water balance and avoided water deficit. The impact of elevated CO_2 on the WUE appeared greater under drought condition. Wullschleger and Norby (2001) demonstrated that 28 per cent increase in WUE was attributed to 15 per cent increase in dry matter increment and 12 per cent decrease in water use. Higher soil water content in elevated CO_2 made itself available to either postpone the onset or reduce the severity of drought. Accordingly this conserved water alone attributed to 20 per cent increase in above biomass extending the photosynthetic active period under moisture stress condition.

Elevated CO_2 brought about a significant increase in net assimilation rate (NAR), relative growth rate (RGR) and crop growth rate (CGR) at each stage of growth in *Brassica*. Higher NAR, RGR and CGR was observed in the cultivar 'RH-30'. The reduction in NAR, RGR and CGR was observed under moisture

stress condition. The adverse effect on NAR, RGR and CGR was ameliorated by elevated CO₂.

Increased CO₂ concentration significantly reduced the leaf area ratio (LAR) and specific leaf area (SLA). Moisture stress has no significant effect on these parameters.

Present study revealed that the elevated CO₂ concentration significantly enhanced the leaf weight ratio (LWR) and specific leaf weight (SLW). A marked increase in LWR and specific leaf weight was recorded in the 'RH-30' cultivar. Moisture stress significantly decreased the LWR and SLW at each stage of growth. The stress-induced reduction in the LWR and SLW was lesser at higher CO₂ concentration.

The promotion of NAR by high CO₂ contributed to elevating RGR, though high CO₂ substantially decreased LAR. This meant that high CO₂ promoted the dry matter partitioning to leaf sheath, stem and root fraction in rice (Imai 1995).

CO₂ has a greater effect on dry matter partitioning. The dry matter partitioning in different parts such as leaf, stem, root and pod were significantly enhanced by CO₂ enrichment. Liu *et al.* (2002) reported that the elevated CO₂ resulted in larger fresh mass, dry mass, leaf area and leaf thickness in two-year old needles of Sitka spruce (*Picea sitchensis*). Tree height, basal diameter and biomass production were also increased, regardless of N supply.

In the present investigation in *Brassica* the increased relative growth rate due to CO₂ enrichment attributed to reduction in LAR and SLA and increase in NAR. The increase in the rates of biomass accumulation and the relative ratio of biomass to leaf area expansion besides increase in the number of leaves may possibly explain this. Woodward *et al.* (1991) also reported that LAR and SLA was significantly higher at ambient than at elevated CO₂ in *Sinapis alba*.

Yelle *et al.* (1990) observed that NAR and SLW in *L. esculentum* and *L. chmielewskii* were higher in CO₂ enriched plants and suggested that assimilate were

preferentially accumulated in the leaves as reserves rather than contributing to leaf expansion. In the present investigation, with *Brassica* also, the compensatory effect of CO₂ to ameliorate the stress effect was highly significant on roots and leaves. This could be attributed to the continuous translocation of photosynthates to these sinks throughout the growth period. Elevated CO₂ brought about a significant alteration in crop growth period. The vegetative, reproductive and total crop growth period was increased due to CO₂ treatment. Moisture stress significantly reduced the vegetative, reproductive and total crop growth period.

The increase of vegetative period indicated that the plant have got sufficient time for development of new branches and leaves. These newly developed leaves and branch act as temporary sinks for utilization of photo assimilates produced in sufficient amount due to CO₂ enrichment. There was no deposition of carbohydrates in the older leaves in the early stages of growth. These newly developed leaves act as source of assimilate for the development of reproductive organs such as flowers and siliquae, which was the strongest sink for utilization of photo-assimilates.

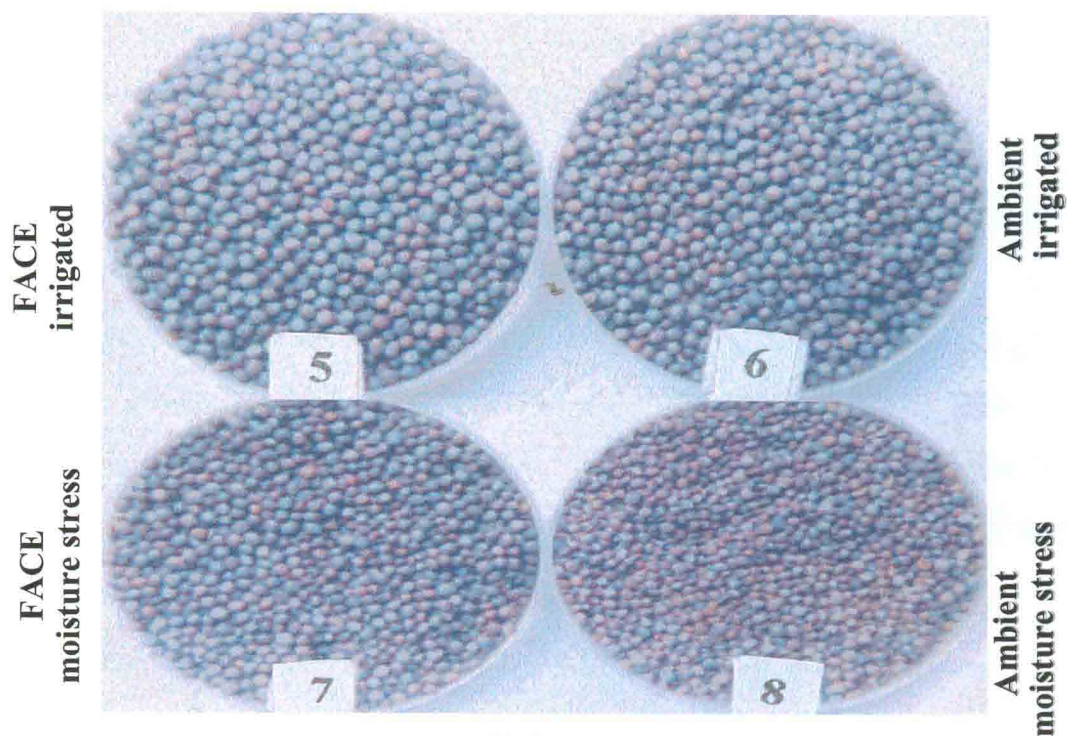
Consequent increase in reproductive period and longer production of photo-assimilates may help in catering the demand for siliquae development through continuous flow of photosynthates, eliminating feedback inhibition. Thus, elevated CO₂ regulated the ontogeny of *Brassica* species in a programmed manner. This process was distinctly superior in 'RH-30' compared to 'Pusa Gold'.

Excess carbohydrates produced at vegetative stage fully utilized in the production of new vegetative structures such as leaves, branches and roots. Similarly at flowering it helped in the development of reproductive parts, whereas, at seed filling this was being utilized for the development of high-density grains (Plate 6).

Carbon dioxide enrichment improved the productivity of *Brassica* spp. by affecting changes in various yield components such as number of pods per plant, seed per pod, seed: husk ratio, 1000-seed weight, sink capacity, yield per m² and



Pusa Gold



RH-30

Plate 6 Effect of elevated CO₂ and moisture stress on seed size of *Brassica* cultivars

harvest index. Greater response for these yield characters was recorded in 'RH-30' compared to 'Pusa Gold'. Moisture stress adversely affected the yield components whereas, the CO₂ enrichment brought about marked amelioration of this stress effect on yield parameters.

Elevated CO₂ induced increase in grain weight and yield per m² may be attributed to the development of numerous siliquae in frequent intervals in different branches to coincide with photo-assimilate production at various newly developed sources. This has helped in catering the demand of newly induced sinks by the excess photoassimilates produced under CO₂ enriched condition. The increase in phloem size observed under the elevated CO₂ in *Brassica* species has helped in the conduction of large amount of photo-assimilate to sink for a larger period to meet their requirement smoothly. It appeared that phloem size developed according to size of sources and sinks. Evans *et al.* (1970) observed that the size of phloem area within peduncle in modern wheat was greater than that of its ancestor 'Dicoccum' and was correlated to greater translocation rate. However, according to Stitt (1991), if the sink strength could not express its full potential and remained low due to technological errors, the accumulation of assimilates may occur in the source leaves resulting in feed back inhibition of photosynthesis.

Baker *et al.* (1990) reported that in rice, number of panicles per plant was entirely responsible for a 47 per cent increase in grain yield under elevated CO₂. Lawlor and Mitchell (1991) squarely attributed the CO₂ induced increase in grain yield to the production of large number of tillers, branches and seed number.

The senescence of leaves also delayed under elevated CO₂ in the present study. Delay in senescence brought about an increase in flowering period and leaf area duration (LAD). This gave additional advantage to the plant for their seed filling and seed development, resulting in bolder grains. It was also observed that the *Brassica* plant grown in higher CO₂ condition flowered 4-5 days earlier and sustained flower initiation for longer period (5-6 days) in comparison to the ambient grown plants indicating that the plant has got more time for sink development and utilization for photoassimilates.

Carbon dioxide enrichment significantly improved the quality of *Brassica* seeds. Reducing, non-reducing, total sugar and starch content were significantly higher in seeds of CO₂ enriched plant. Moisture stress adversely altered the reducing, non-reducing, total sugar and starch content in the *Brassica* seeds. The stress induced adverse effect of these parameters was significantly ameliorated by CO₂ enrichment. CO₂ enrichment brought about significant reduction in nitrogen and protein content of the seeds. Similarly, moisture stress also reduced the nitrogen and protein content in the seeds. There was no significant difference in terms of nitrogen and protein content under drought in elevated CO₂ condition. In the present study, the higher C : N ratio observed in seeds of CO₂ enriched plant. Moisture stress significantly reduced the C : N ratio. This adverse effect was significantly depressed by CO₂ treatment.

The present study clearly indicated the sustainance of the stimulatory effect of CO₂ up to seed formation stage. The sequestration of carbon in plant parts lead to the accumulation of greater carbohydrate components in *Brassica* seeds. The stress-induced reduction in carbohydrate was greatly reduced in elevated CO₂, possibly due to greater photosynthate transfer to seeds. The greater proportion of nutrients (CHO) of the grains was observed due to the redistribution of carbon from the vegetative parts to the seeds. The sequestration of additional carbon possibly helped in compensating the adverse moisture stress effects on seed carbohydrate component. Although oil content in *Brassica* seed was significantly reduced by moisture stress but this reduction was markedly ameliorated by elevated CO₂. This result was in accordance with the findings of Uprety *et al.* (1997) in *Brassica juncea* seeds. According to them, the stress induced adverse effects on grain composition particularly on carbohydrate and oil content significantly improved due to sequestration of carbon and improved water status. The accumulation of carbohydrates possibly resulted in reduction in protein content due to dilution effect. According to Conroy (1992) the reduced transpiration may also contribute to the reduction in nitrogen uptake resulting in lower protein content in harvested *Brassica* seeds (Conroy 1992).

The CO₂ enrichment significantly increased the oil content in the seeds of *Brassica* spp. Moisture stress reduced the oil content of *Brassica* cultivars. The stress induced adverse effect on reduction in oil content was ameliorated by CO₂ enrichment. Significant increase in oil content attributed to participation of additional CO₂ in stimulating the activity of acetyl CoA enzyme (Uprety *et al.*, 1997) which increased the formation of abundant malonyl coA under elevated CO₂ condition and these steps play a positive role in regulating the fatty acid biosynthesis.

The elevated CO₂ significantly altered the fatty acid composition of seeds (Fig. 42a & 42b). The palmitic, steric, oleic, linolenic and erucic acid significantly reduced due to CO₂ enrichment. The linoleic acid was increased. There was no significant difference between cultivars for their fatty acids contents, however, significantly higher amount of oleic acid was observed in 'RH-30' cultivar.

It was demonstrated that the elevated CO₂ brought about a reduction in the saturated fatty acids, palmitic, stearic acid and mono-unsaturated fatty acids such as oleic acid content indicating that most of the fatty acid undergo desaturation and produced unsaturated fatty acid due to higher CO₂ effect. Significant higher quantity of linoleic acid was found in seeds of CO₂ enriched plant. The conversion of oleic acid to erucic and linoleic acid to Linolenic acid requires sufficient quantity of O₂ that was not available due to higher intercellular concentration of CO₂ which inturn, resulted in reduction of polyunsaturated fatty acid. Although conversion of oleic acid to linoleic acid was also a desaturation process, in that step optimum level of O₂ may be available for desaturation. After large consumption of O₂ in this step, sufficient inter cellular O₂ was not available for the subsequent desaturation. Moreover, at this point ratio of CO₂ : O₂ ratio increased significantly due to high consumption of O₂, which ultimately reduced the rate of de-saturation process (Fig. 43). Another possibility of reduction of linoleic acid was that most of the linolenic acid was used for chloroplast membrane organization (Thiew, 1970).

Alteration in fatty acid composition will be one of most important consequence in changed climate due to the rise in atmospheric CO₂ in near future.

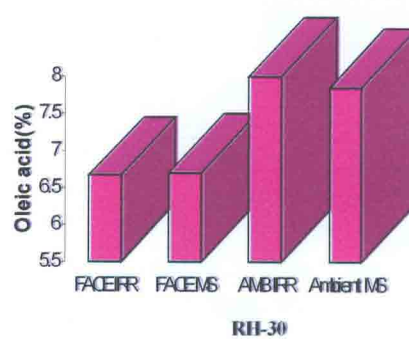
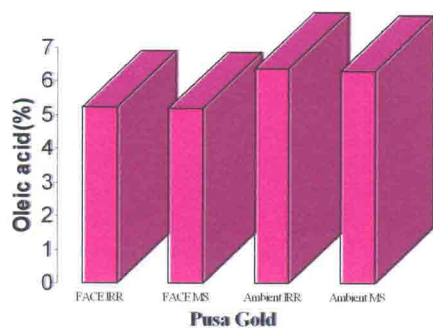
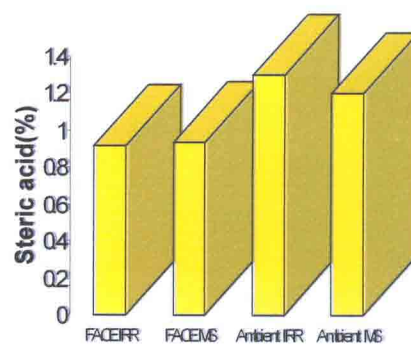
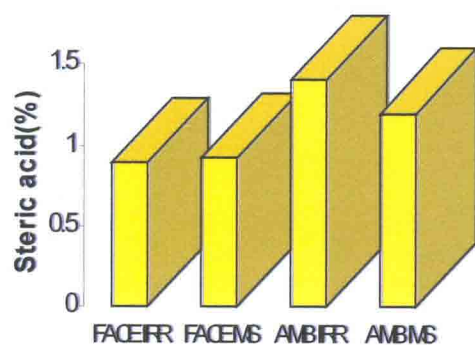
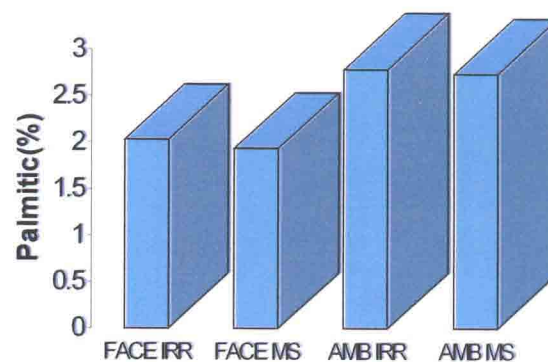
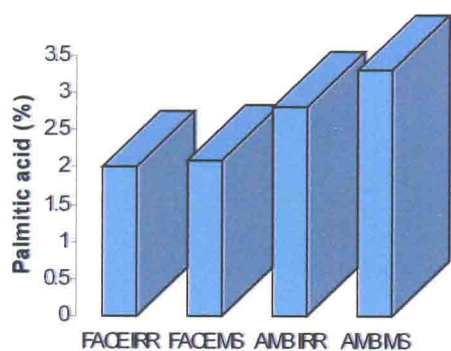


Fig 42a. Interactive effect of elevated CO₂ and moisture stress on fatty acid composition in *Brassica* seeds

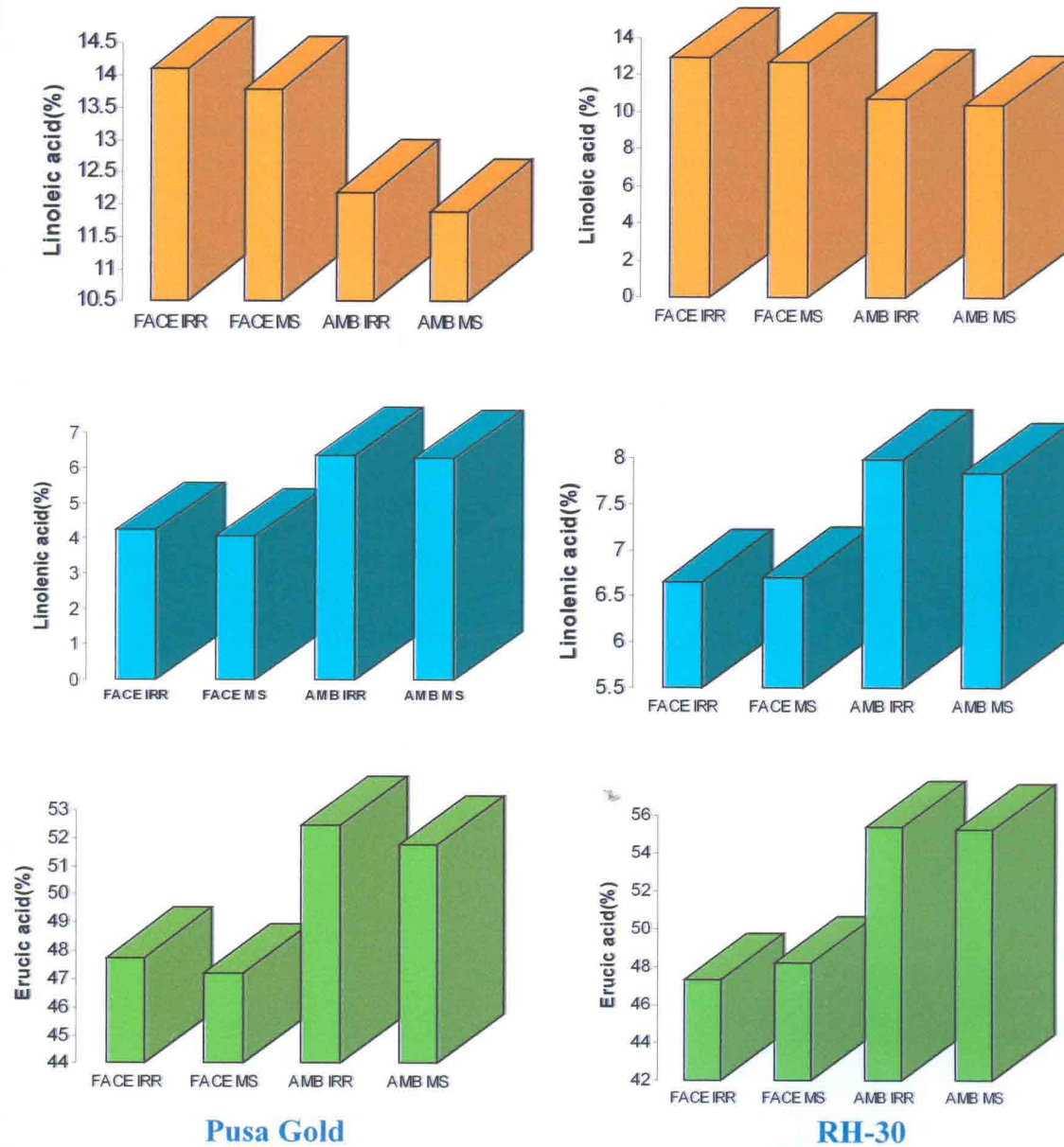


Fig. 42b Interactive effect of elevated CO₂ and moisture stress on fatty acid composition in *Brassica* seeds

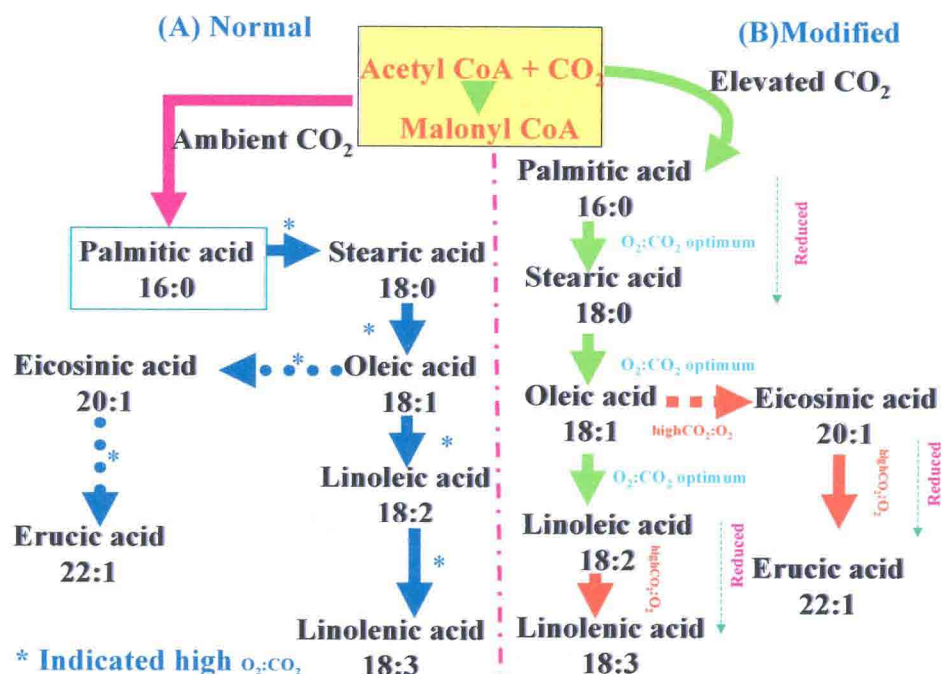


Fig 43. A= Normal pathway of fatty acid synthesis in *Brassica* seeds (Stumpf 1983); *indicated that the O₂: CO₂ ratio was high during biosynthesis of fatty acids. B = CO₂ induced modification: Optimum O₂:CO₂ ratio up to the biosynthesis of i. linoleic acid. ii. reduction in O₂:CO₂ during biosynthesis of (a) linoleic acid to linolenic acid (b) oleic acid to erucic acid

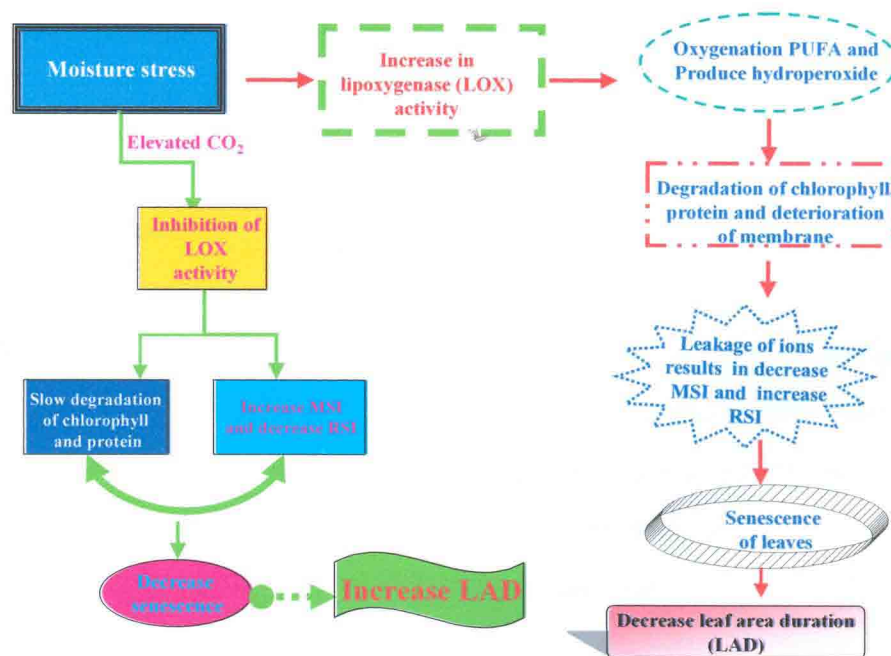


Fig 44. Mechanism of CO₂ induced delay in leaf senescence

Reduction of saturated fatty acid pool and some unsaturated fatty acid like linolenic and erucic acid are important from quality point of view because most of these fatty acids increase the blood cholesterol in human body; however, saturated and mono-unsaturated fatty acids are less involved in this.

CO₂ enrichment brought about a reduction in the crude fiber content in seeds. The decrease in crude fiber may be attributed to the incorporation of most of the carbon to the fatty acid synthesis rather than carbon based polymers and fiber. According to Getinet *et al.* (1996) decrease in crude fiber contents may have resulted from high grain weight, which was positively correlated with oil and negatively correlated with crude fiber content.

Elevated CO₂ significantly increased the water status of the leaves in *Brassica* by affecting water relation parameters. The water potential, osmotic potential, and relative water content were increased significantly in leaves of *Brassica* cultivars throughout the growth period. Moisture stress adversely affected these water relation parameters. The stress-induced effect on these parameters significantly ameliorated in higher level of CO₂.

Water status observations indicated that the stress effect on leaf water status may be countered due to increase in the random distribution of water molecules and more free available water for participation in metabolic activities under elevated CO₂ condition, which was confirmed by Nuclear Magnetic Resonance (NMR) studied. NMR offers a non-destructive and non-invasive method for characterizing tissue water status (Mathur-DeVre, 1979). Colire *et al.*, (1988) have shown that spin-lattice relaxation time (T_1) of leaf water was closely related to classical water status parameters such as RWC, water potential and solute potential in barley leaves. According to them, it determines the internal water availability, its physiological compartmentation, its states, and the variations under stress. The higher T_1 implies a more random distribution of water molecules and free availability of water for participation in metabolic activities (Lewin, 1974). In the present study, T_1 increased significantly in leaves of *Brassica* cultivars throughout

the growth period. Higher-level proton spin-lattice relaxation time (T_1) was observed in 'RH-30' cultivar. Moisture stress decreased the proton spin-lattice relaxation time and this reduction was lesser under higher level of CO_2 . Upreti *et al.* (1995) also demonstrated that elevated CO_2 partially ameliorated the negative effects of moisture stress in *Brassica* spp. by increasing the water potential, higher apoplastic water and regulating turgor through positive changes in tissue elasticity. In the present investigation, the water status effected more through changes in the dynamic phase of water (water potential and mobility of water molecules) compared to static phase i.e. relative water content.

Centritto *et al.* (1999) attributed the delay in the onset of drought under elevated CO_2 to the decline in stomatal conductance, reduction in transpiration, and also to increase in plant water potential. These componental changes were responsible to maintain the higher plant water potential in drying soils enabling plants to remain turgid and functional for longer time.

The sequestration of carbon due to elevated CO_2 resulted in the greater root growth, secondary root number, and root volume in *Brassica* cultivars in the present investigation. The increase in these parameters helped in the absorption of available water. Increased root volume indicated that more water and nutrient could be absorbed. Several reports (Charves and Pereira 1992 and Moya *et al.*, 1998) indicated that elevated CO_2 promoted root proliferation and increased exploitation of deep soil moisture reserves in both temperate and tropical species, minimising or delaying the impact of drought. According to Pritchard and Rogers *et al.* (2000), atmospheric CO_2 enrichment can change the distribution of roots in the soil, which might also have implications for water uptake. Several experiments suggested that crop plants in higher CO_2 atmosphere will have larger root systems that were highly branched, especially at shallow depth, and this should increase the capacity for resource acquisition, but at lower efficiency CO_2 enrichment of a grassland community led to an upward shift in root length density with greater proportion of roots in the top 6 cm soil layer. This shift was perhaps related to increased soil moisture and its effect on nutrient availability (Arnone *et al.*, 2000).

Thus, increased absorption of water from soil due to greater root growth and maintenance of leaf water status by simultaneous reduction in transpiration due to decreased stomatal conductance are the major consequence of elevated CO₂ in *Brassica* species under moisture stress condition. These important adjustments helped in protecting them from the adverse stress effect. Apparently, the higher turgor pressure and the maintenance of greater water potential under elevated CO₂ was sufficient to produce normal cell elongation in the leaf and stem, sustaining the optimum plant growth which otherwise would have been retarded by moisture stress. These results well correlated to the changes in the observed water status of the plant and could either support the hypothesis that under drought, the high CO₂ improved the water status of plants due to increased stomatal resistance, reduced transpiration and greater uptake of water due to enhanced root growth or could be explained by improvement in the capacity of the plant photosynthetic system to use higher CO₂.

Besides adjusting the regulation of water in plants through changes in various water relation components, CO₂ enrichment also helped in osmotic adjustment in *Brassica* species under moisture stress condition.

The accumulation of soluble sugars as well as non-structural carbohydrates, free amino acids, proline and free fatty acid in the cell-sap possibly helped in the maintenance of osmo-regulation in *Brassica* leaves under moisture stress condition. The higher levels of carbohydrates play a protective role against the photo-assimilate shortage during stress. These may contribute to the ameliorating effect of elevated CO₂ to drought stress responses in the present investigation. Read *et al.* (1997) demonstrated that the CO₂ induced accumulation of fructans in canola did not interfere with the photosynthetic apparatus and may possibly used for osmoregulation. According to Ellsworth (1999) elevated CO₂ may directly alter leaf dehydration tolerance in cases where CO₂ induced excess carbohydrates serve as osmotica. Elevated CO₂ could increase drought tolerance of plants if increased rates of net carbon assimilation leads to an increased availability of substrate for osmotic

adjustment. Elevated CO₂ could therefore, have significant effect on drought tolerance by inducing carbohydrate accumulation for growth and by enhancing osmotic adjustment during drought (Wullschleger *et al.*, 2002).

Carbon dioxide enrichment did not affect the proline and free amino acid content in *Brassica* species. Moisture stress significantly increased the proline and free amino acid content. Significantly higher amount of both these compounds were observed in elevated CO₂ condition under drought. The proline and free amino acid content was several fold higher in elevated CO₂ compared to ambient under drought. The proline and other free other amino acids may also possibly play an important role in the osmo-regulation under stress condition.

Elevated CO₂ brought about a significant increase in free fatty acid content in the leaves of *Brassica* cultivars throughout the growth period. Moisture stress significantly increased the free fatty acid content. The free fatty acid content was significantly increased under drought in elevated condition. They were released by the action of lipolytic enzymes in leaves of *Brassica oleracea* plant when subjected to water stress (Cheour *et al.*, 1992) and involved in metabolic adjustment during stress (Aziz and Larher, 1998).

Accumulation of substrates such as H₂O₂ and MDA responsible for oxidative stress was significantly decreased due to CO₂ enrichment in *Brassica* cultivars, whereas, moisture stress brought about significant increase in these intermediates. Similar trend was also observed for lipoxygenase enzyme activity. Elevated CO₂ ameliorated the stress induced increase in these oxyradicals and lipoxygenase enzyme activity.

Lipoxygenase are a class of non-heme, iron-containing dioxygenase that catalyse the oxygenation of polyunsaturated fatty acid with conjugated diene hydroperoxide. Lipoxygenase are responsible for the formation of hydro-peroxide (MDA) to induce membrane deterioration. Both lipid peroxidation and hydroperoxide promote losses of membrane integrity, protein and chlorophyll resulting in

yellowing of leaves (Yamuchi *et al.*, 1986). The inhibition effect of elevated CO₂ on these oxy-compounds and lipoxygenase activity helped in delaying the senescence of leaves (Fig. 44).

Zhuang *et al.* (1994) also suggested that LOX mediated lipid peroxidation might be involved in chlorophyll degradation. The delayed senescence of *Brassica* leaves in the present investigation can be explained from sustenance of chlorophyll pigments activity for longer time due to the depression in LOX activity and their intermediates. CO₂ enrichment significantly increased the membrane stability index resulting in lowered relative stress injury.

Higher level of membrane stability index and depressed level of relative stress injury was recorded in 'RH-30'. Moisture stress significantly reduced the membrane stability index resulting in the enhancement of relative stress injury. However, CO₂ partially reversed these stress effects. The delay in senescence intensity was one of the most important consequences of increasing leaf area duration and seed filling period. Increased leaf area duration indicated that plants have got more time for the production of photosynthate, which was ultimately used for seed development.

Brassica is an indeterminate type of crop plant. The flowering and fruit development took place in different branches at different time. The unsynchronised ontogeny of reproductive structures was one of the distinct characters of *Brassica* plant. Therefore, crop required longer period of leaf area duration with higher rate of photosynthesis for better yield. The increased leaf area duration coupled with more photosynthetic rate during seed filling was observed in the present study resulted in the greater productivity of the *Brassica* cultivars under elevated CO₂ conditions. These results indicated that plants significantly modified their ontogenic events to take advantage from the changed environment.

Moisture stress also causes oxidative damages due to the increased production of potentially toxic radicals such as O₂⁻ and H₂O₂. Such an effect of oxidative stress was markedly altered due to elevated CO₂ in *Brassica* cultivars.

It was revealed that the increased concentration of CO₂ significantly decreased the antioxidant enzyme activity like superoxide dismutase, catalase peroxidase, ascorbate peroxidase, and glutathione reductase in the leaves of *Brassica* plants throughout the growth period. The higher activity of these enzymes was recorded in 'RH-30' cultivar.

The activity of these enzymes increased in both elevated and ambient condition under moisture stress. But activity was many fold higher in elevated CO₂ under moisture stress condition. The entire enzyme system was involved in scavenging of the Reactive Oxygen Species (ROS) produced during any kind of stress.

In order to minimize cellular oxidative damage, plants produce antioxidative enzymes including SOD, CAT, POD, and AOPD. Exposure to elevated CO₂ significantly affected the activity of antioxidative enzymes. The significant decrease was observed in the activity of these enzymes because of decrease in cellular ROS production (Pritchard *et al.*, 2000). This reduction may reflect in oxidative stress due to CO₂ enrichment. The reasons for decreased oxidative stress effect was:

- (a) Increase in CO₂ : O₂ ratio within chloroplast, which may decrease electron leakage from PSI to O₂ (Fig. 45).
- (b) Increased CO₂ : O₂ ratio would decrease oxygenase activity of Rubisco reducing photorespiration and resultant cellular H₂O₂ production.
- (c) Stimulation of antioxidants activities under elevated CO₂ in moisture stress condition (Fig. 45).

Foyer *et al.* (1994) also demonstrated that formation of ROS at low chloroplastic CO₂ concentration in higher plants. Another factor increasing the ROS production was the drought-induced reduction in respiration rate. Such stress induced the production of oxyspecies, which damage the plant cell by oxidizing membrane lipids besides affecting the proteins, chlorophyll and nucleic acid (Foyer *et al.*, 1994).

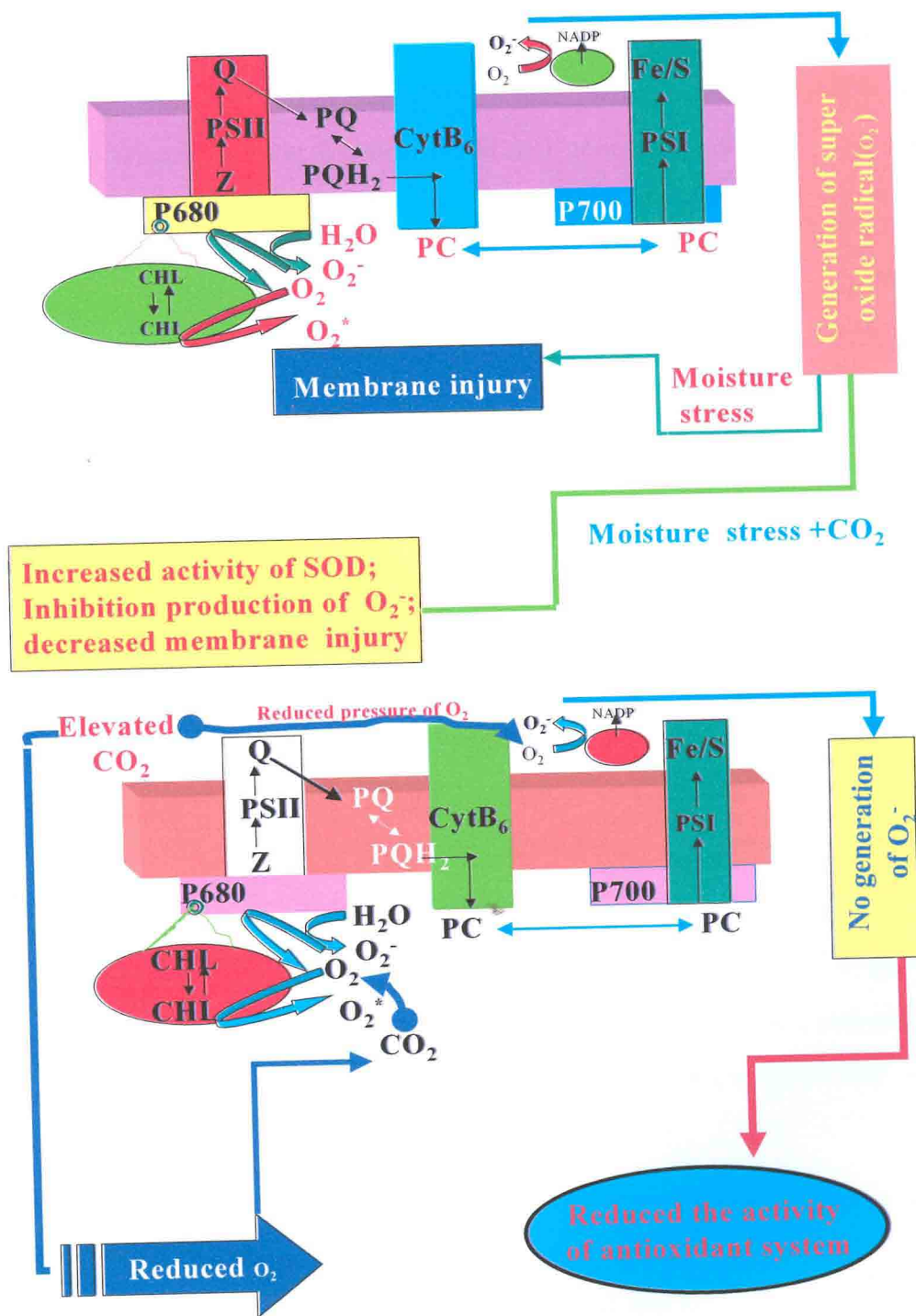


Fig 45: Production of super oxide radicals and singlet oxygen in chloroplast at the site of PSI and PSII (modified from Foyer *et al.* 1990, Harbison *et al.* 1999). This pathway was modified under elevated CO₂: Chloroplastic O₂: CO₂ ratio reduced resulting in the reduced in the formation of oxyradicals.

In the present study, it was demonstrated that the reduction in the entire antioxidant enzyme system caused by the elevated CO_2 did not allow *Brassica* plants suffer from oxidative stress. Sufficient intercellular CO_2 was available to utilize the reducing power by reducing it and generation of ROS. This result was in accordance with the observation of Polle *et al.* (1993) in *Picea abies* L. According to Pritchard *et al.* (2000), constitutive antioxidative enzymatic activities may decrease under high CO_2 condition because of decrease in cellular ROS production. Significant reduction in the activity of SOD, POD, CAT, AOPD and GR was observed in the plants grown under elevated CO_2 . The up-regulation or down-regulation of plant antioxidant system depended upon the extent of oxidative stress, CO_2 and genotype interactions (Pritchard *et al.*, 2000 and Polle *et al.*, 1997). Badani *et al.* (1993) reported that increasing atmospheric CO_2 enhanced the demand for photo-reducing power and this could reduce risk of oxyradical formation in soybean. The development of drought injury symptoms may in part be due to the production of activated oxygen species (AOS) such as superoxide radicals, singlet oxygen, H_2O_2 and hydroxyl radicals. Superoxide radicals are detoxified by SOD. H_2O_2 was destroyed by catalase and different kind of peroxides and ascorbate glutathione cycle (Ascorbate peroxidase and glutathione reductase) (Asada, 1994).

Possibly the elevated CO_2 weakened electron transport from monodehydroascorbate to ascorbate. Doubling CO_2 lowered the extra-chloroplastic H_2O_2 production by depressing photo-assimilation and reduces the risk of oxygen toxicity associated with photosynthetic electron flow.

Although elevated CO_2 decreased the whole antioxidant system, the significant increase in entire antioxidant enzymes were observed under moisture stress. It reduced the respiration significantly and altered the O_2 : CO_2 ratio resulting in to the generation of ROS. However, high CO_2 helped for quick induction of the antioxidative system to scavenge the ROS. Schwanz *et al.* (1996) also found that the elevated CO_2 significantly reduced activities of some enzymes in both pine and oak. According to them, the rate of O_2^- production was increased in plants

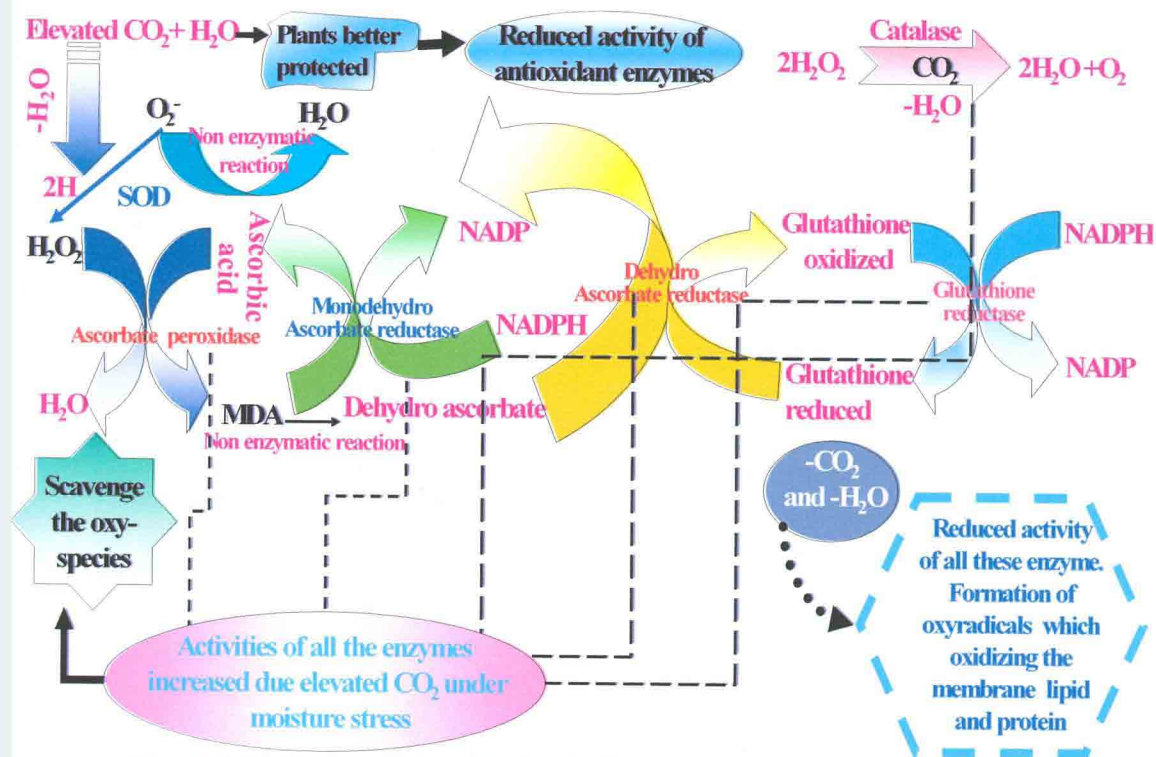


Fig. 46. indicating scavenging role of antioxidant enzymes to the interaction of moisture stress and high CO_2 (modified from Asada- Halliwell pathway)

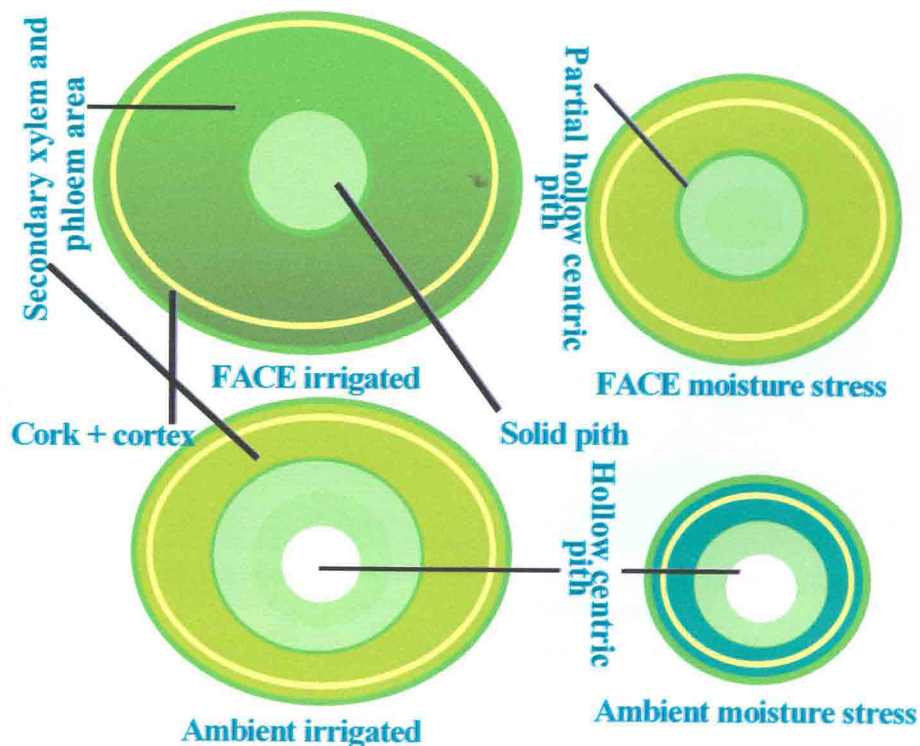


Fig. 47 Diagrammatic presentation of transverse section of main shoots of *Brassica* species

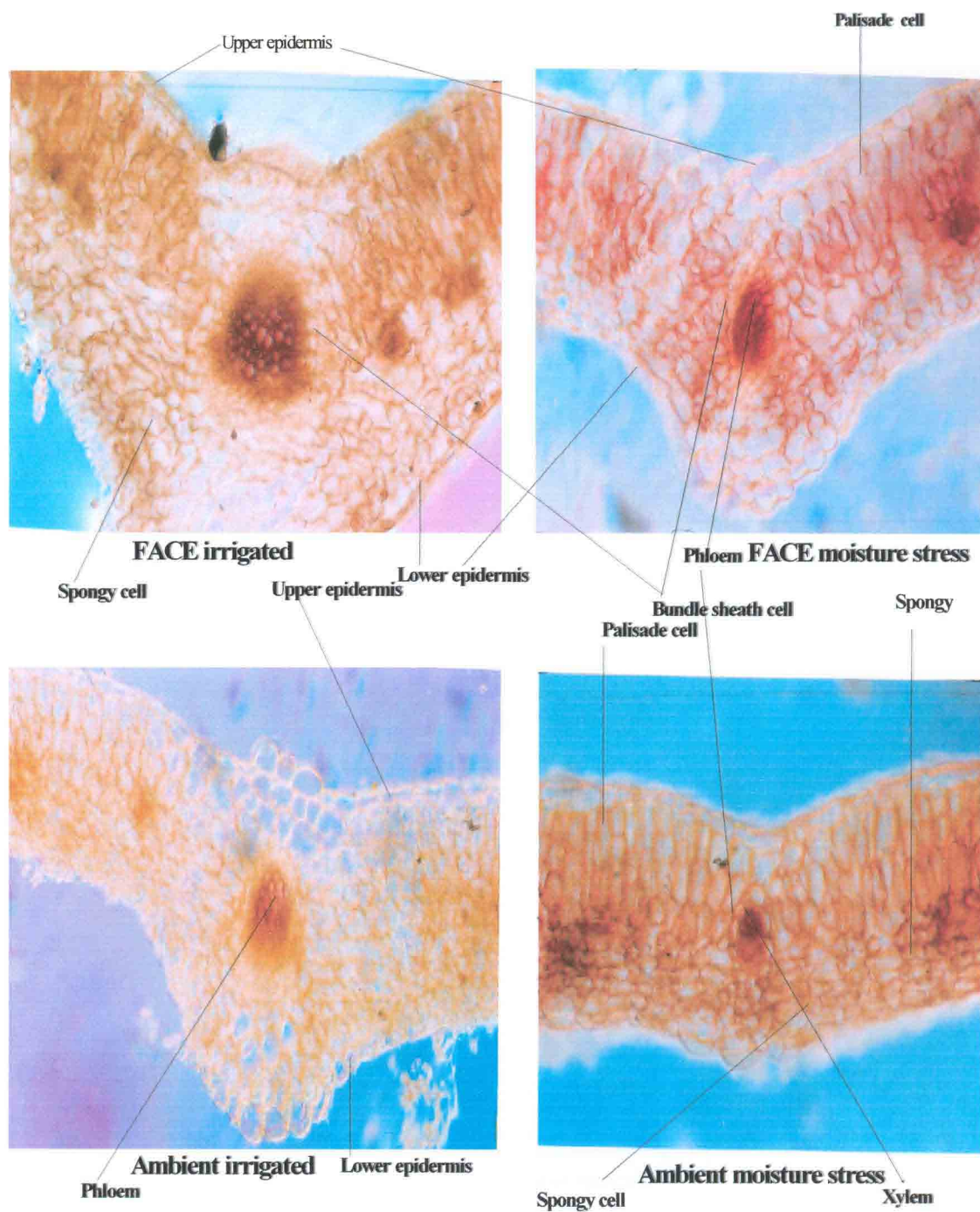


Plate 7 Effect of elevated CO₂ and moisture stress on root anatomy of *B. campestris* cv Pusa Gold (LM 100x)

grown at elevated CO_2 when subjected to drought. Perhaps plants grown at an elevated CO_2 concentration contain increased metabolic reserves to respond to oxidative stress compared with plants grown at ambient CO_2 concentrations. Present study on *Brassica* showed higher activity of antioxidant under drought in elevated CO_2 condition than the ambient drought. It was apparent that the plants have their own systems for scavenging superoxide radicals. Superoxide dismutase (SOD) catalysed the disproportionation of O_2 to H_2O_2 and O_2^- . H_2O_2 was decomposed by catalase or ascorbate peroxidase (APX), the latter enzyme oxidized ascorbate to monodehydroascorbate (MDA), which was then re-reduced by NAD(P)H in a reaction catalysed by monodehydroascorbate reductase (MAR) (Fig. 46). Increase in the size of glutathione pool and total superoxide dismutase activity in oak leaves under the water shortage and CO_2 enrichment was considered by Marabottini *et al.* (2001) as the compensatory effort by plant.

Carlson and Bazzaz (1982) demonstrated that plants grown with elevated CO_2 might be better protected from environmental stresses by an increased capacity for detoxification and repair. This type of detoxification also prevailed in *Brassica* cultivars in the present study. The stress-induced enhancement of H_2O_2 and malondealdehyde (MDA) significantly depressed under elevated CO_2 indicating the significant enhancement in antioxidant enzyme pool in *Brassica* plant. Thus *Brassica* cultivars developed strategy to combat the stress induced oxidative damage on cell membrane both by reducing reactive oxy-species and increasing antioxidant enzymes.

Elevated CO_2 brought about increase in leaf thickness, which was also more prevalent in 'RH-30'. It also greatly ameliorated the stress-induced reduction in leaf thickness. The increase in the palisade and spongy layers along with that of epidermal cell layers contributed to the total leaf thickness under elevated CO_2 (Plate 7 & 8).

Increased CO_2 concentration has no significant effect on cell size and number, in spongy cells. Moisture stress significantly decreased the cell size and number in spongy layer.

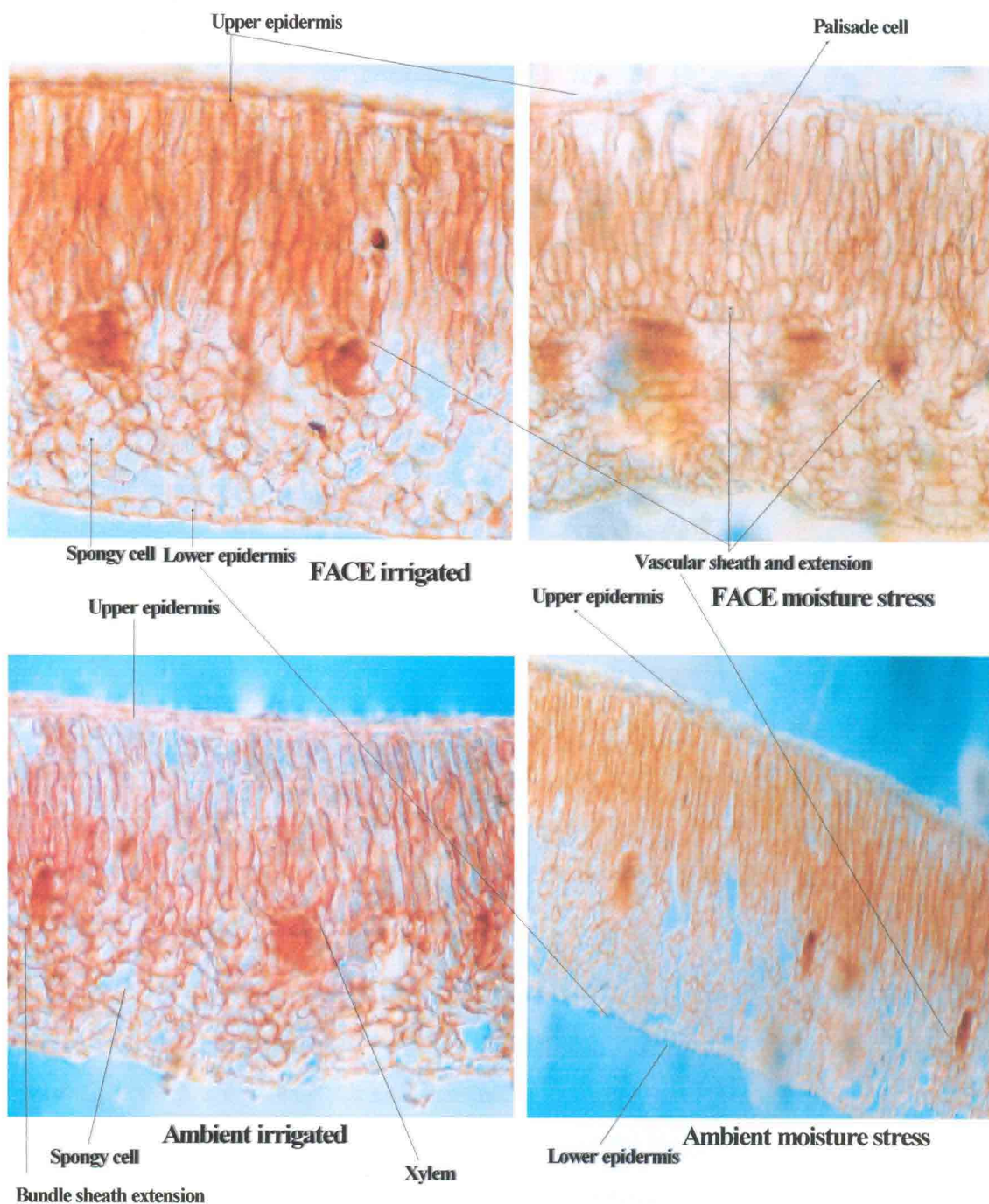


Plate 8 Effect of elevated CO₂ and moisture stress on leaf anatomy of *Brassica juncea* cv. RH-30 (LM 100x)

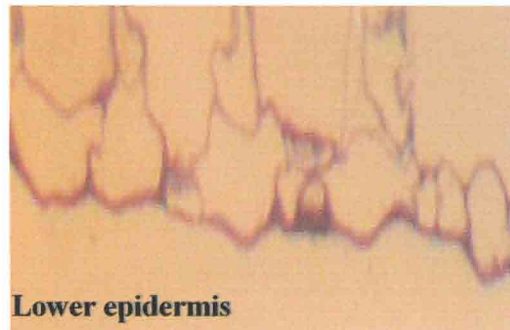
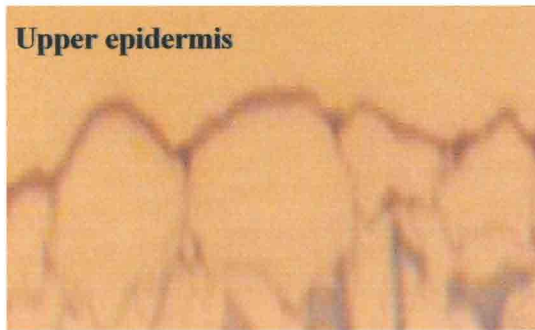
The increase in epidermal layer (Plate 9 & 10) was one of the most important strategy of plant for amelioration of moisture stress effect because the thicker epidermis may reduce the rate of transpiration through the accumulation of cuticular wax. These results were in conformity with that of Thomas and Harvey (1983) in *Glycine Max* and Lee *et al.* (1993) in *Betula pendula*.

Ferris and Taylor (1996) in *Populus* demonstrated that carbon induced growth effect occurred because of the enhanced wall extensibility and the effect of wall loosening enzyme, xyloglucan endotransglycosylase (XET). Masale (2000) and Zhuo *et al.* (2000) observed increased supply of soluble carbohydrates stimulated both cell wall expansion and cell production but the mechanism remained elusive. Ferris *et al.* (2001) in their FACE experiment with *Populus* species found that in *P. nigra* leaf expansion was due to the production of larger cells whereas, for *P. alba* production of smaller cells with increase in XET activity in both the species.

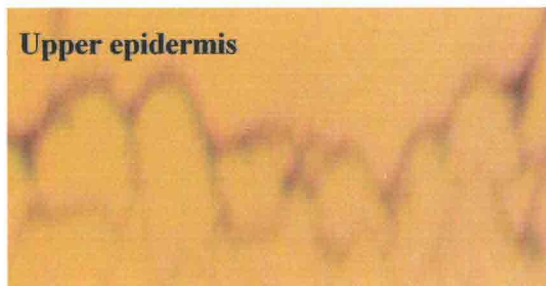
According to Thomas and Harvey (1983), differences in thickness were due to the result of increased palisade at initiation and development. Elevated CO₂ induced one more layer of palisade cells in the mesophyll and greater cell expansion in *Brassica* leaves in the present investigation. This resulted in the increased osmotic potential of leaf cell due to higher carbohydrate content resulting into more absorption of water.

The CO₂ enrichment brought about significant decrease of stomatal density in the leaves (abaxial and adaxial), stem and siliquae of *Brassica* cultivars. Similarly, moisture stress markedly reduced the stomatal density in these organs. The reduction in stomatal density was significantly more in elevated CO₂ under drought in leaves and siliquae although higher reduction was observed in stem.

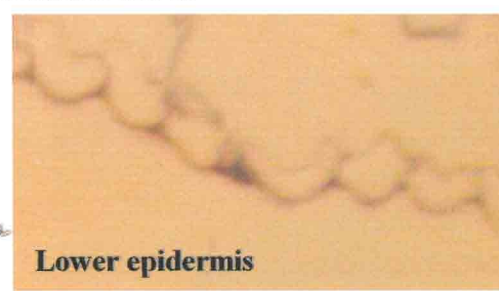
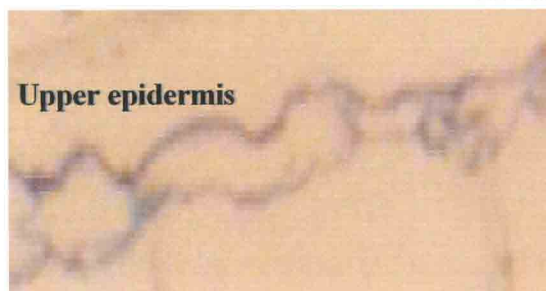
The complete opposite trend was observed in case of stomatal index (abaxial and adaxial). CO₂ enrichment significantly increased stomatal index in the leaves, stem and siliquae. The moisture stress decreased it. The stress-induced reduction was ameliorated in elevated CO₂ condition.



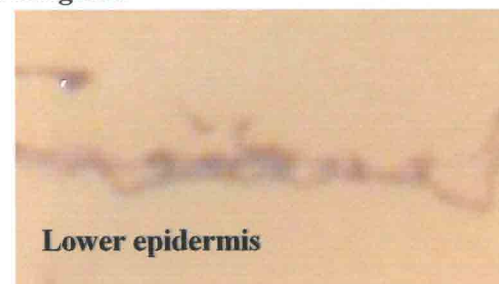
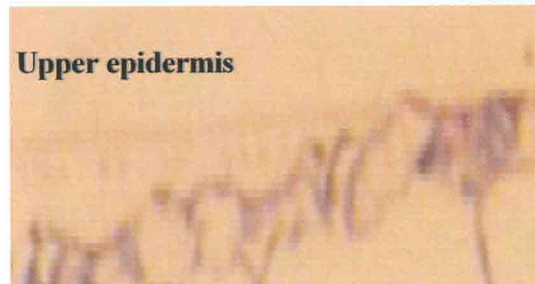
FACE irrigated



FACE moisture stress

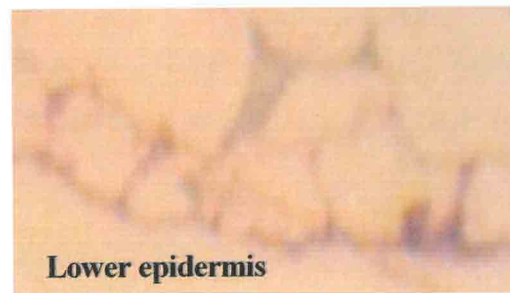
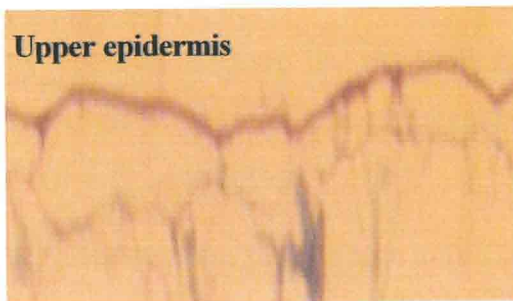


Ambient irrigated

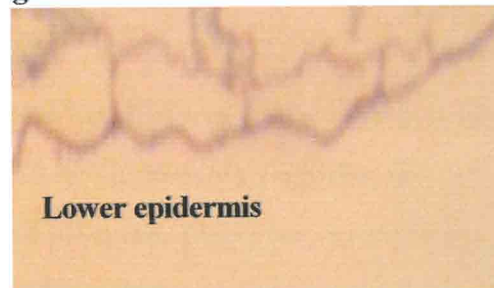
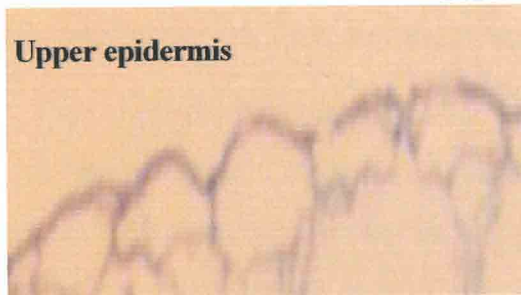


Ambient moisture stress

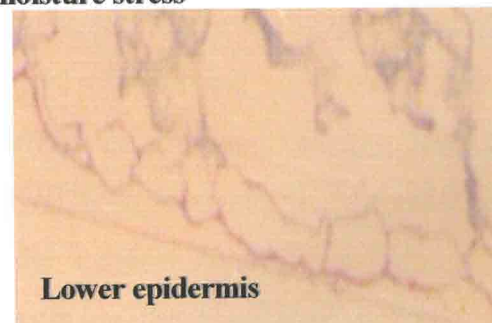
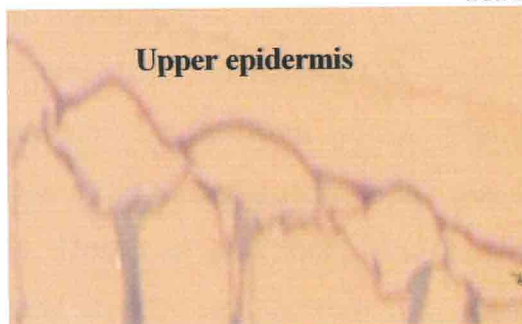
Plate 9 Interactive effect of CO₂ and moisture stress on leaf epidermis of *Brassica campestris* cv. Pusa Gold (LM100x)



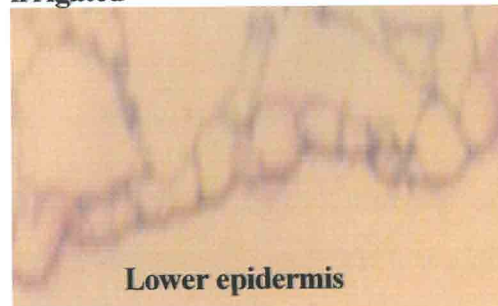
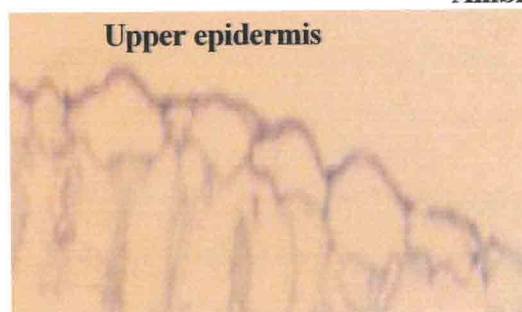
FACE irrigated



FACE moisture stress



Ambient irrigated



Ambient moisture stress

Plate. 10 Interactive effect of CO₂ and moisture stress on leaf epidermis of *Brassica juncea* cv. RH-30 (LM 100x)

The CO₂ enrichment markedly increased the stomatal size in terms of length, breadth and area in leaves (abaxial and adaxial) stem and siliquae. The moisture stress markedly reduced the stomatal size in leaves (abaxial and adaxial) stem and siliquae. Adverse stress effect on stomatal characters was markedly lesser under elevated CO₂ condition.

The stomatal pore length was significantly higher in elevated CO₂ condition in leaves (abaxial and adaxial) stem and siliquae. The reduction in stomatal pore length was observed in moisture stress condition.

An increase in stomatal index and consequent decrease of stomatal density due to the elevated CO₂ has been attributed to increase of epidermal cell and subsequent decrease of stomata in leaves and siliquae. However, in stem the CO₂ has no effect on epidermal cell differentiation thus stomatal density was not altered by the interaction of elevated CO₂ and moisture stress. These findings also indicated the site-specific response of CO₂ under different climate and give further research opportunity for in-depth study. The reduction in stomatal density ultimately decreased the transpiration and increased the WUE in *Brassica* (Rabha and Uprety, 1998). Uprety *et al.* (2000) observed that the increase in leaf size affected the stomatal density in four rice cultivars by reducing the irradiance and spreading stomata differentially. They demonstrated that CO₂ induced reduction in stomatal conductance, increased stomatal index, size of stomatal guard cells, stroma and epidermal cells, made rice cultivars to adjust to elevated CO₂ environment.

Madsen (1973) attributed the reduction in stomatal density of tomato leaves to CO₂ induced decline in leaf nitrogen status. According to Ticha (1982), the effect on stomatal density was a consequence of increase in the size of epidermal cells due to greater turgor. Han *et al.* (1999) reported that increased CO₂ concentration reduced average stomatal opening, stomatal conductance and stomatal density to 78 per cent, 80 per cent and 87 per cent, respectively, compared with their values under normal ambient CO₂ in *Pinus sylvestris*. Increased CO₂ also reduced transpiration to 76 per cent, thus increasing the WUE of the plants.

In the present study, with *Brassica* the length and width of epidermal cells were increased (Plate 11 & 12, LM and Plate 13 & 14 EM) at high CO₂ condition, indicating greater effect on cell expansion, which was associated with increase in osmotic potential due to accumulation of saccharides. These changes along with those in stomatal characters helped in regulating the reducing transpiration, gas exchange and regulating osmotic movement of cell sap to maintain turgor under moisture stress condition.

The elevated CO₂ significantly altered the vessel characters of shoot and root. The vessel numbers, size (length, width and transactional area) were increased due to the CO₂ enrichment. All these characters adversely affected by moisture stress. Stress effects on these anatomical features were partially reversed due to the elevated CO₂.

Significant alteration in both meta- and proto-xylem and phloem characters were observed in shoots and roots due to elevated CO₂. It was noted that elevated CO₂ significantly reduced the pith size due to increase in area and number of secondary xylem and phloem (Plate 15 & 16 and Plate 17 & 18) (Fig. 47). This alteration in xylem and phloem anatomy may increase the stem and root girth. Increased vessel transactional area was an important contributing factor for increasing stem girth.

The increase in radial width of xylems (meta + proto) and phloem (meta + proto) increased under elevated CO₂ (Plate 15 & 16 and Plate 17 & 18) indicated more transfer of water and nutrient from soil to plants resulting in higher water status maintained throughout the growth period and increase in phloem size helped to transfer all the solute material from source to sink. The proto-phloem sieve element solely imports solute for growth (Esau 1978). Meta-phloem functions primarily to transport solute to other developing regions of plant. (Hayes *et al.*, 1985 and Wood *et al.*, 1994). The increase in both the phloem size indicated this modification for unloading of carbohydrates to sink region following increase in sink size (number of siliquae/plant) and sink capacity. The co-existence of two

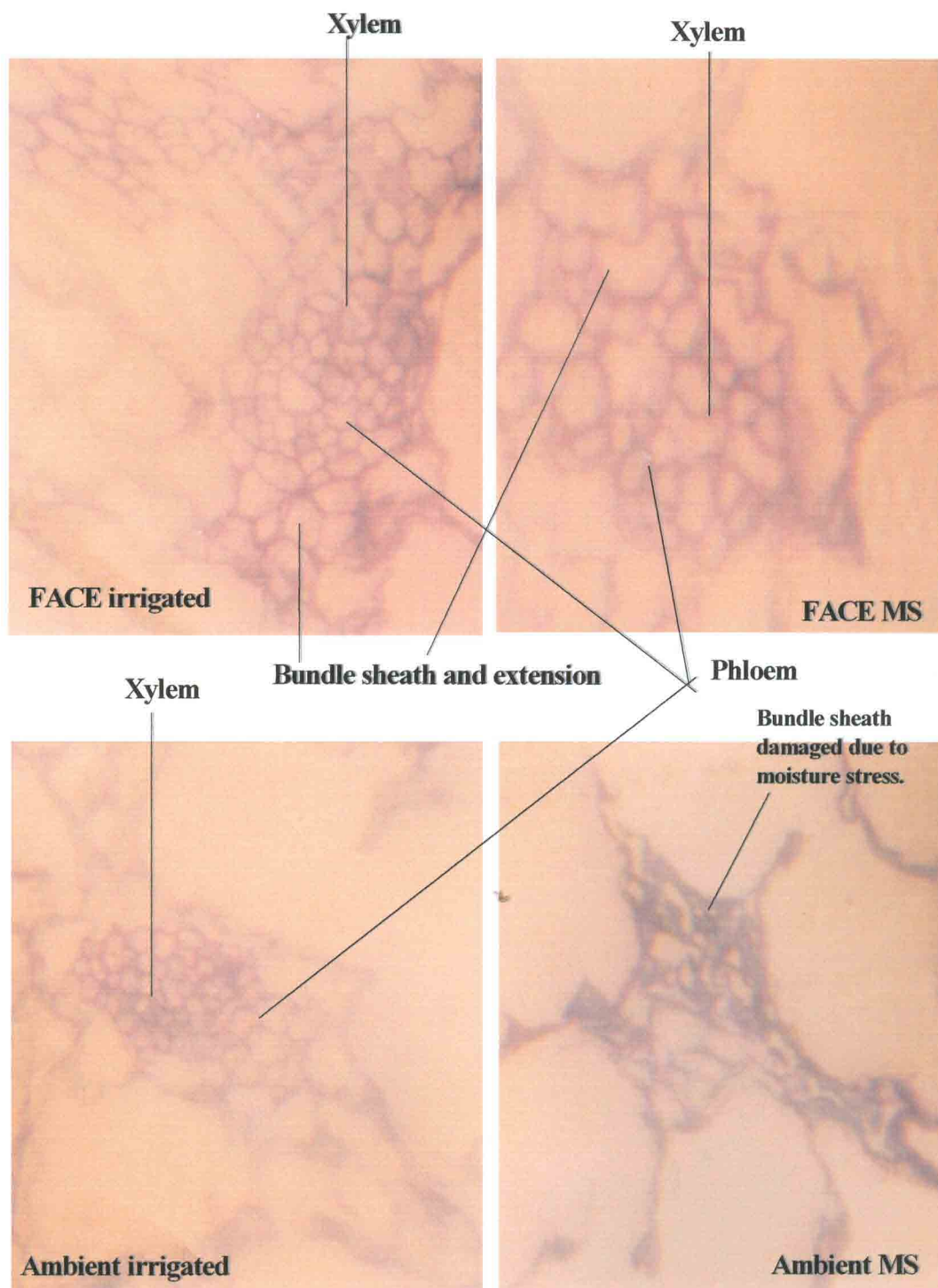


Plate. 11 Interactive effect of CO₂ and moisture stress on vascular bundle of *Brassica campestris* cv. Pusa gold (LM 100x)

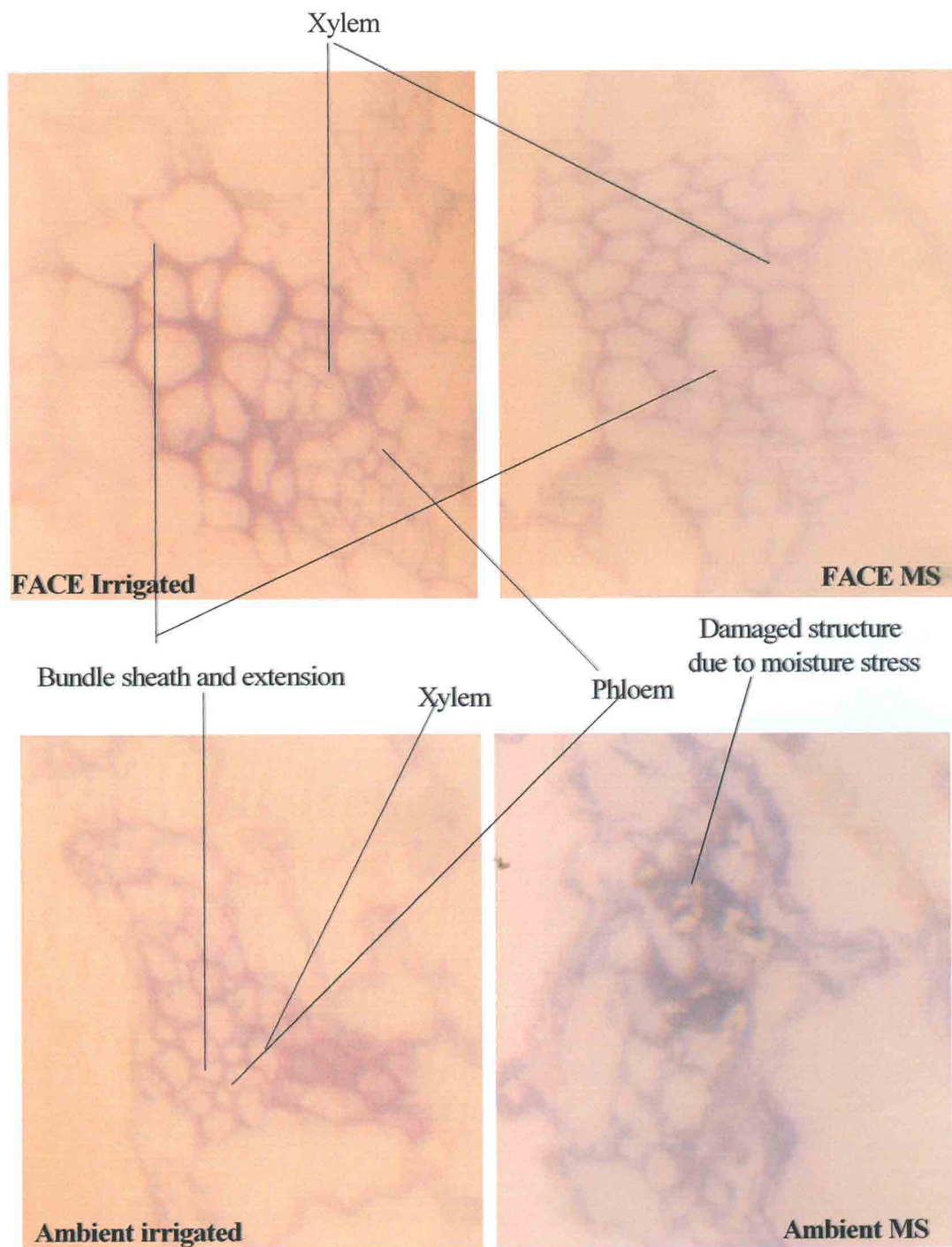


Plate. 12 Interactive effect of CO₂ and moisture stress on vascular bundle of *Brassica juncea* cv. RH-30 (LM 100x)

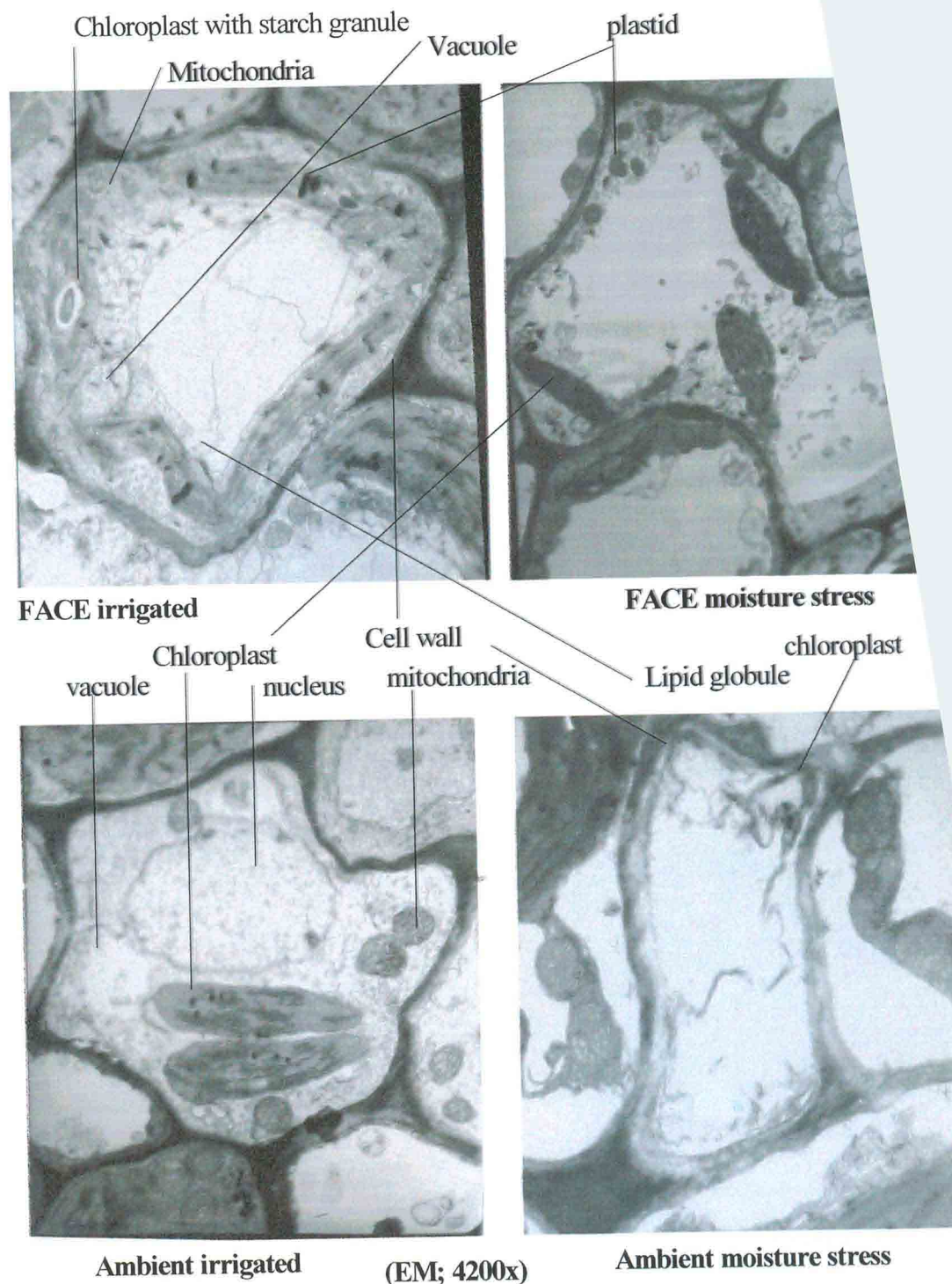


Plate. 13 Effect of elevated CO₂ and moisture stress on vascular bundle cell size and number of chloroplast in the leaves of *Brassica campestris* cv. Pusa Gold

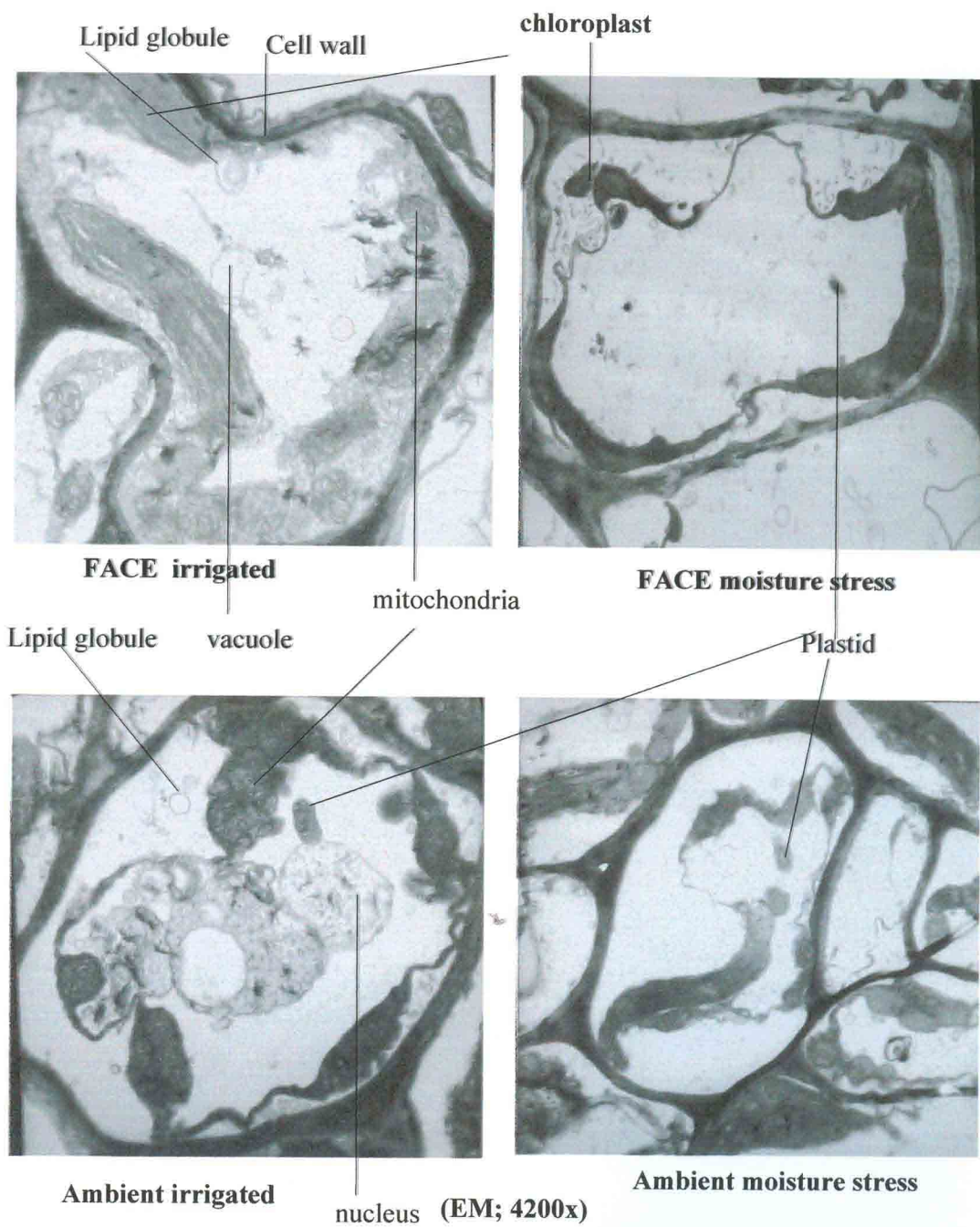


Plate 14 Effect of elevated CO₂ and moisture stress on vascular bundle cell size and number of chloroplast in the leaves *Brassica juncea* cv. RH-30

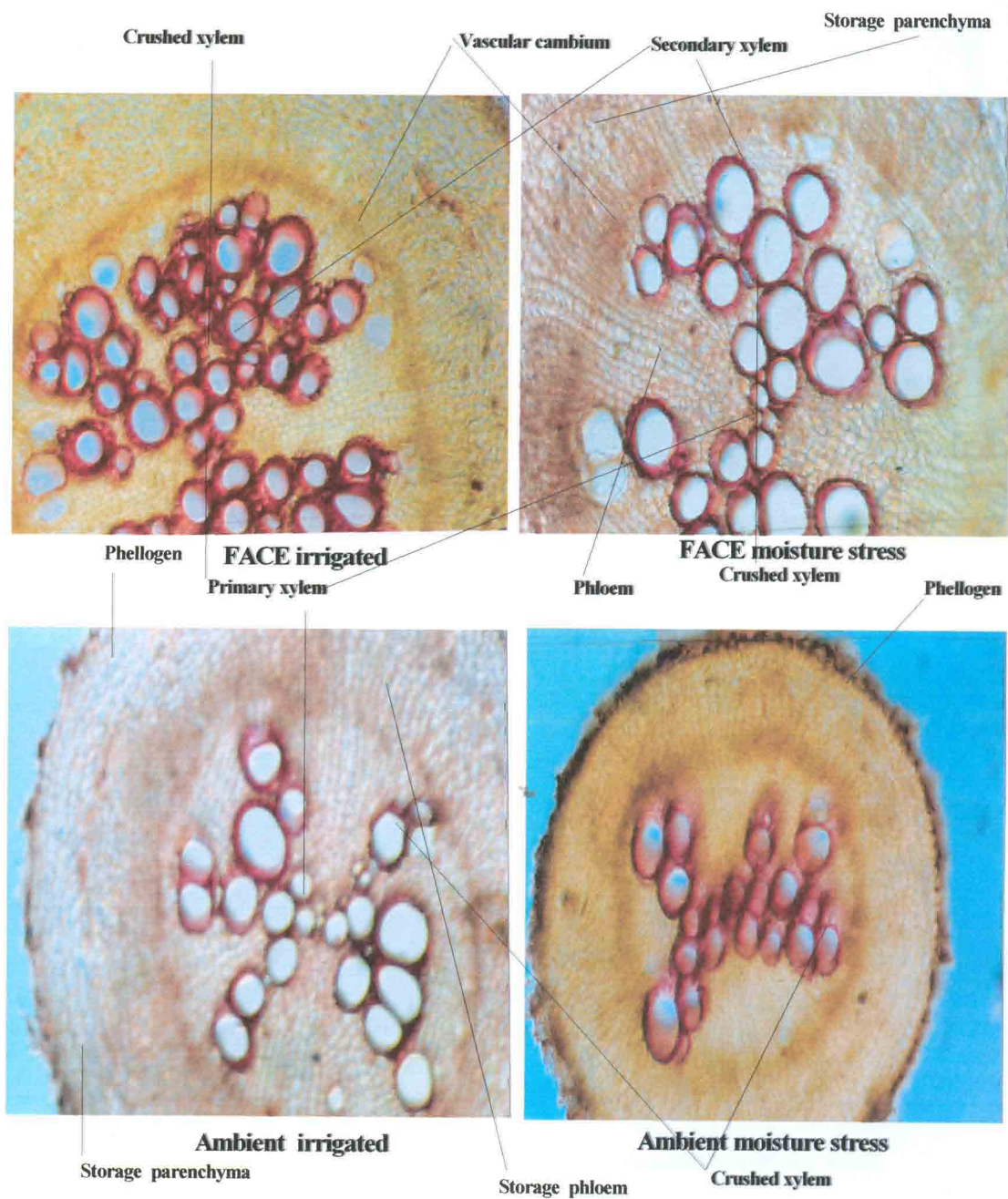


Plate 15 Effect of elevated CO₂ and moisture stress on root anatomy of *B. campestris* cv Pusa Gold (LM 100x)

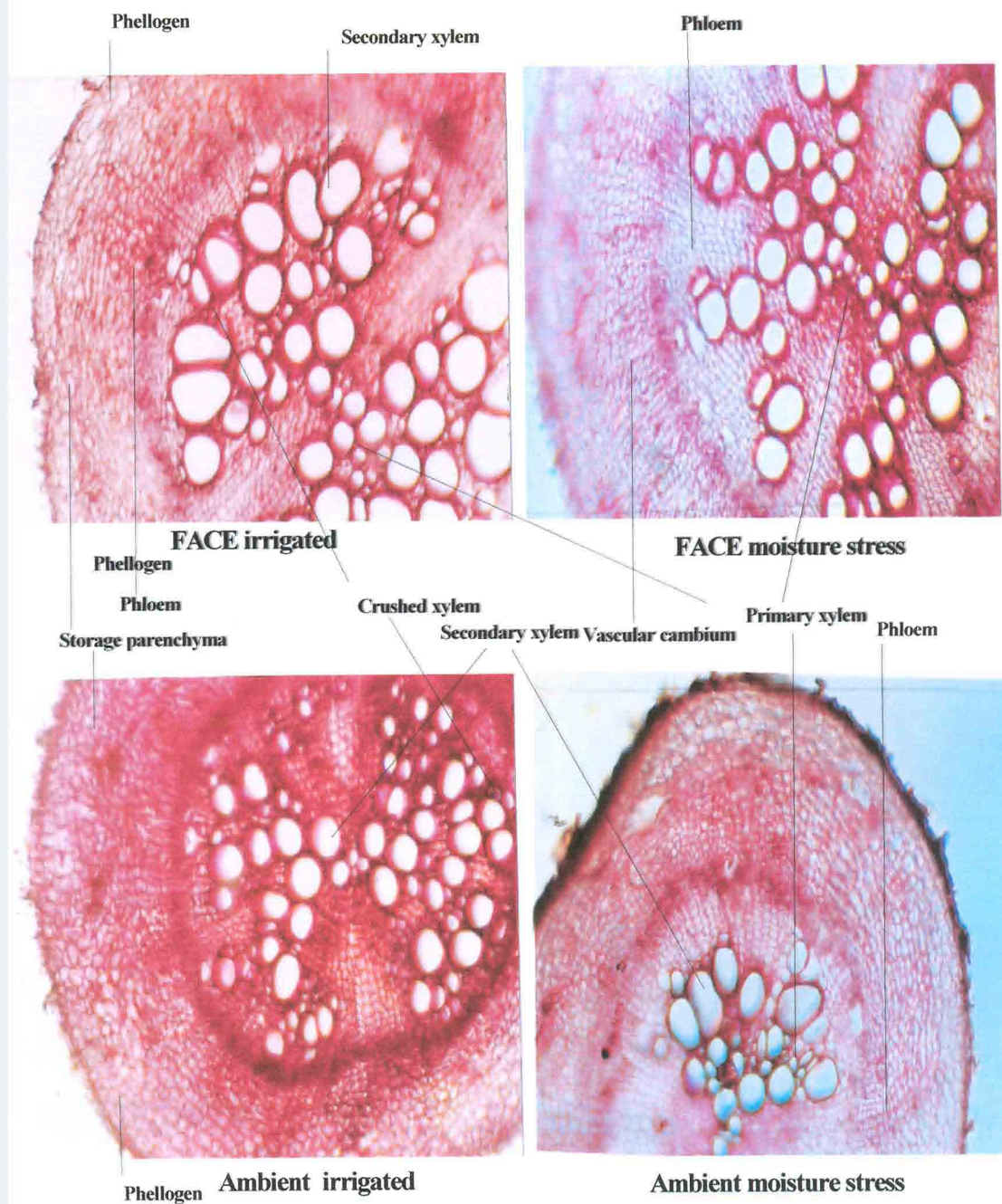


Plate 16 Effect of elevated CO₂ and moisture stress on root anatomy of *Brassica juncea* cv RH-30 (LM 100x)

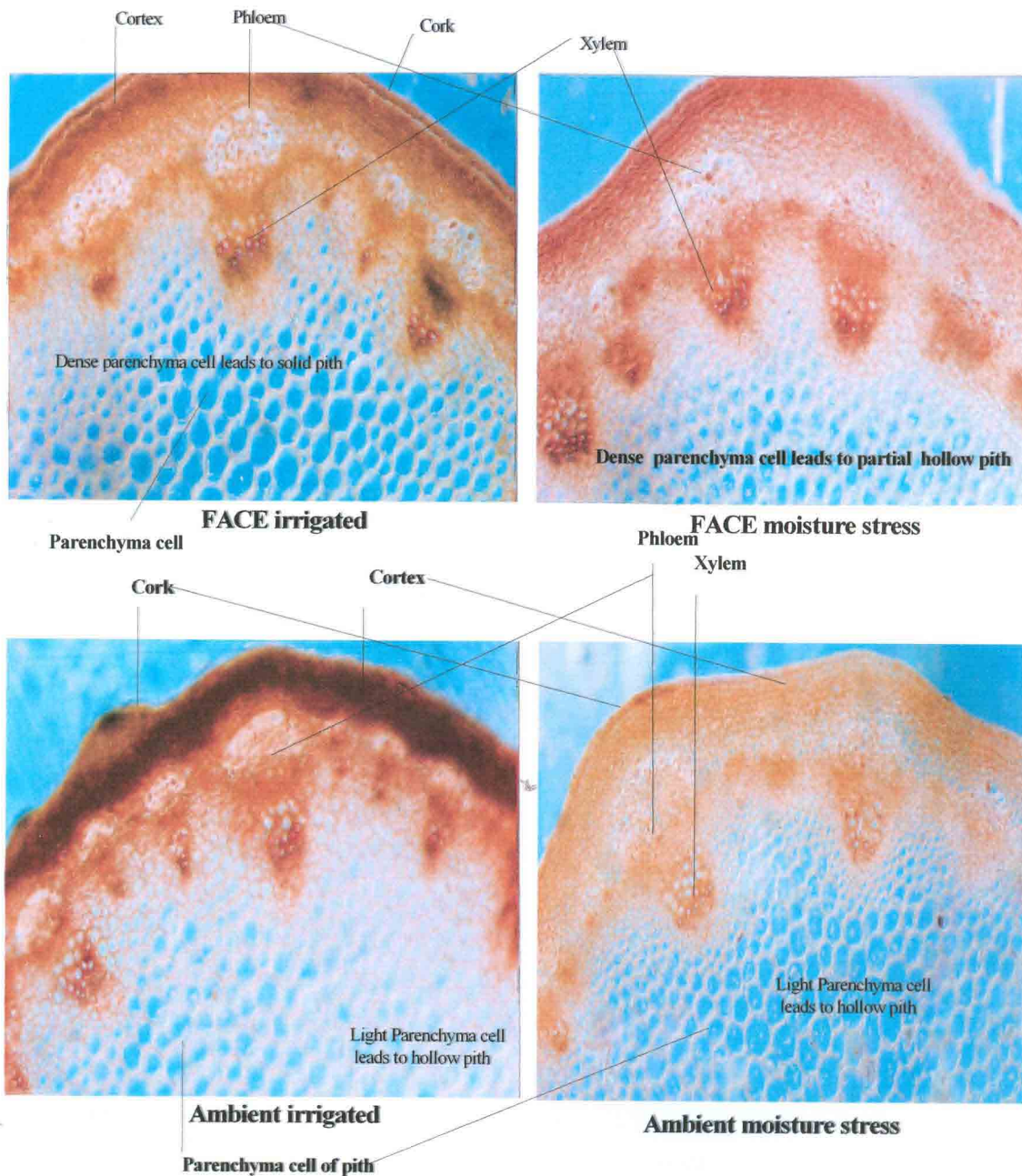


Plate 17 Effect of elevated CO₂ and moisture stress on stem anatomy of *B. campestris* cv Pusa Gold (LM 40x)

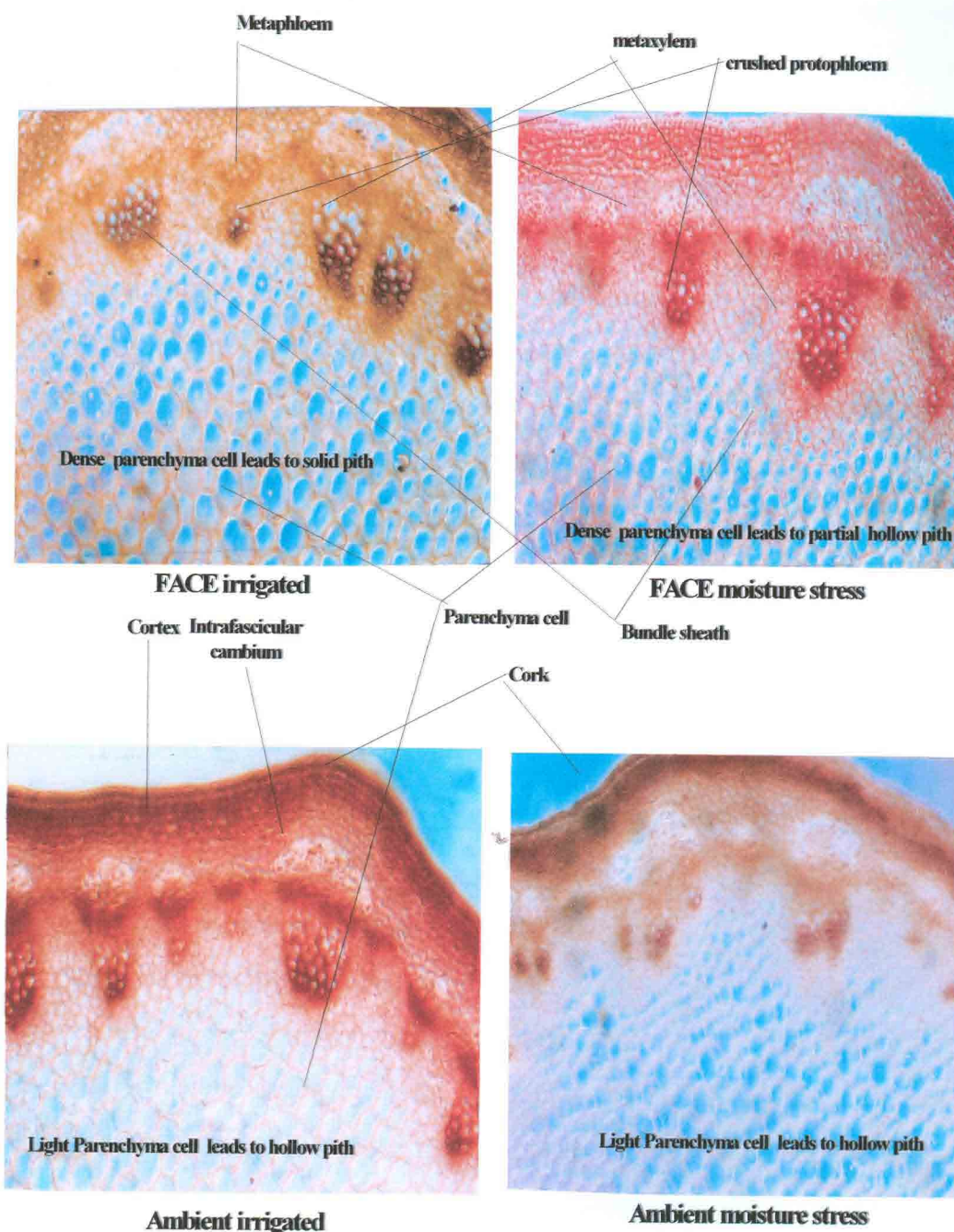


Plate 18 Effect of elevated CO₂ and moisture stress on stem anatomy of *Brassica juncea* cv RH-30 (LM 100x)

ontogenetically different phloem sieve element types in *Brassica* plant under elevated CO₂ indicated the possibility of two different transport functions at the same time (a) One may be involved in supplying for the structural development (leaf size, stem girth and root volume) and (b) Another may cater to the need of increased new sinks.

This indicated that metabolite adjustment through phloem was one of the important area of the future study. Another important consequence of greater phloem size and number in roots which could channelise basipetal movement of the larger amount of carbohydrate to the roots leading to the development of more number of secondary roots, increased root length and root volume in *Brassica* spp. under elevated CO₂ condition.

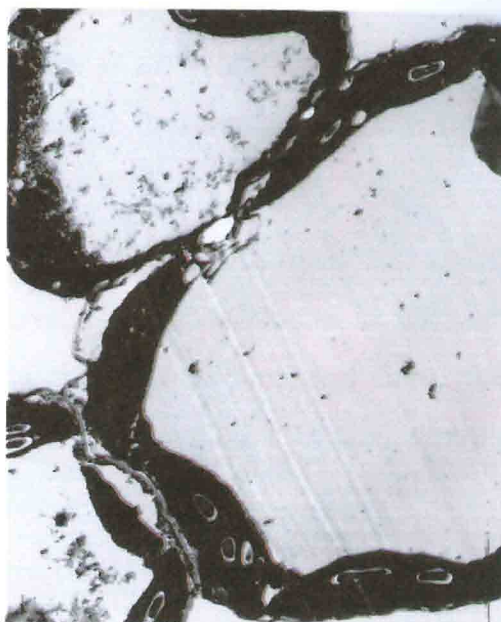
It was apparently seen that elevated CO₂ caused the formation of solid pith under irrigation and partially solid pith under drought. The hollow pith was found in the stem of control plants. Elevated CO₂ depressed the degeneration of parenchyma cells during ontogenic development of tissue. The solid pith was consisted of parenchyma cells.

The CO₂ enrichment significantly increased the chloroplast number in both mesophyll and vascular bundle sheath of the cell. These were markedly decreased under the moisture stress condition. The stress induced reduction in chloroplast number significantly ameliorated in elevated CO₂ condition. Mesophyll cell had more number of chloroplast compared to the vascular bundle sheath cell (Plate 13 & 14 and 19) and laying near the cell wall under elevated CO₂. The increased chloroplast number could relate with increase in the cell size. These findings indicated that cells have got more space to develop number of chloroplast.

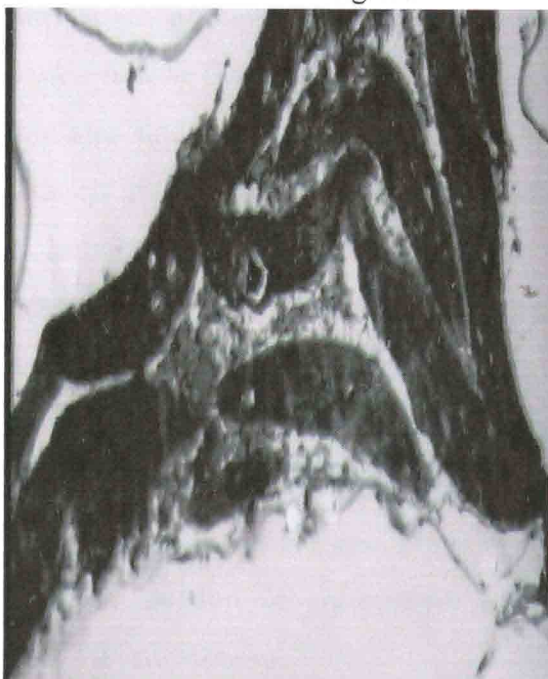
CO₂ enrichment brought about significant increase in the starch granule number within the chloroplast of both mesophyll and vascular bundle sheath cell. There was no significant difference in terms of cultivars. Moisture stress decreased the starch granule number within chloroplast. Starch granule size was larger in mesophyll cell compared to vascular bundle sheath cells.



FACE irrigated



FACE moisture stress



Ambient irrigated



Ambient moisture stress

Plate 19 Effect of elevated CO_2 and moisture stress on number of mesophyll cell chloroplast in the leaves of *Brassica juncea* cv. RH-30. (EM; 2050x)

Robertson *et al.* (1995) observed that the cell and chloroplast expansion was accelerated by 10 and 25 per cent respectively in *Triticum aestivum* cv. Hereward leaves under elevated CO₂. Utriainen *et al.* (2000) reported that CO₂ enrichment caused a significant increase in needle cross-sectional width, and an accumulation of starch in the mesophyll tissue of Scots pine seedlings.

Chloroplasts in leaf tissues of plants from CO₂ enriched treatments appeared denser and were more deeply stained by crystal violet. Changes in chloroplast density or volume, which occurred in, plants grown in CO₂ enriched environment, have been generally attributed to increased starch accumulation in the chloroplasts (Madsen, 1971).

The Transmission Electron Microscope (TEM) study of leaf tissues showed a significant increase in the thickness of epidermis, size of mesophyll cells, accumulation of starch and size and number of starch granules per chloroplast in *Brassica juncea* (Upreti *et al.*, 2001). According to them, the production of photo-assimilates under elevated CO₂ might have increased the sink capacity to adjust the starch granules in the chloroplast without disturbing the thylakoid membrane. The important phenomenon observed in the present study showed that CO₂ enrichment not only increased chloroplast size but it also strengthened the chloroplast membrane (Plate 20 and Fig. 48). The elevated CO₂ increased the starch granule size (Plate 23 & 24) and deposited them near the chloroplast membrane stretching without any damage (Plate 21). Due to deposition of starch granule near the membrane, thylakoid and grana were protected from mechanical damages in the middle portion of chloroplast and ultimately avoid feedback inhibition by metabolite adjustment.

The increased membrane stability index with significant decrease in relative stress injury supported the protection mechanism developed in the plant for chloroplast structure and starch granule.

Plants exhibited improved carbon use efficiency resulting from increased photosynthesis, light use efficiency and decreased dark respiration. The magnitude



FACE irrigated

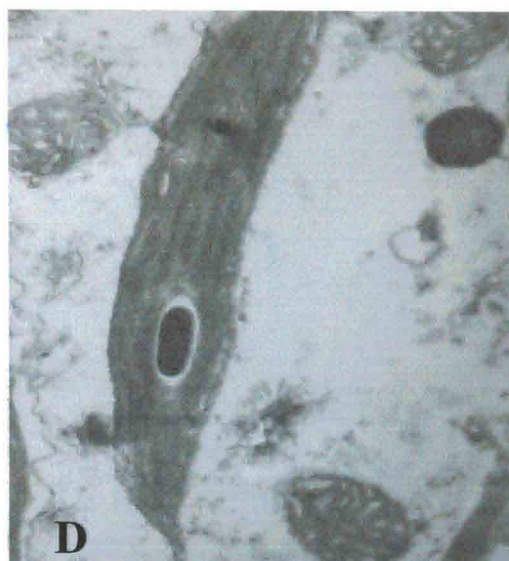


FACE moisture stress

A & B: Deposition of starch granule near the membrane by stretching without any damage to chloroplast machinery under elevated CO₂



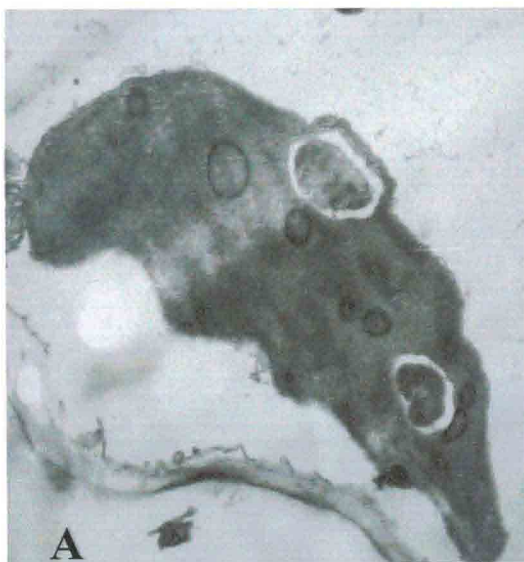
Ambient irrigated



Ambient moisture stress

C & D: Normal deposition of starch granule within the chloroplast

Plate. 20 Effect of elevated CO₂ and moisture stress on starch granule in the leaves of *Brassica campestris* cv. Pusa Gold (EM 8400x)

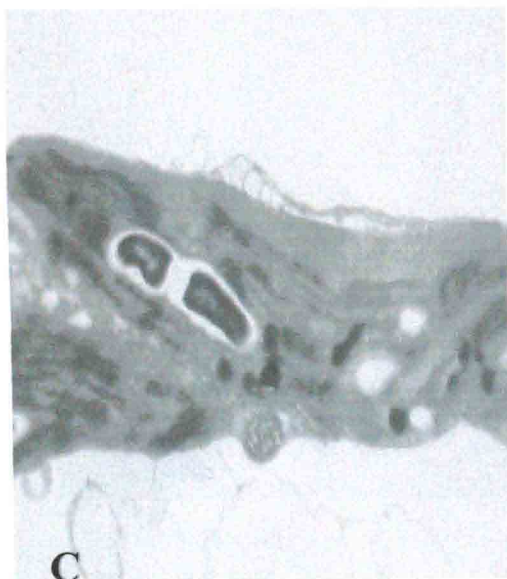


FACE irrigated



FACE moisture stress

A & B: Deposition of starch granule near the membrane by stretching without any damage to chloroplast machinery under elevated CO₂



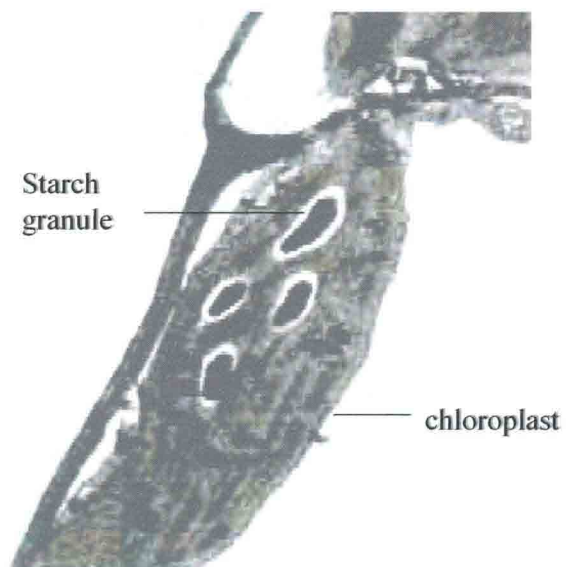
Ambient irrigated



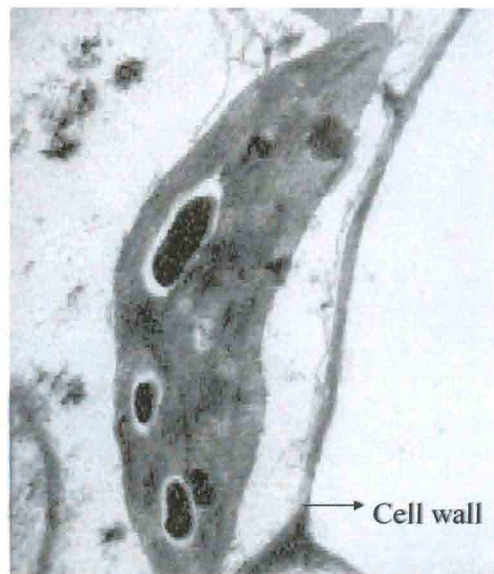
Ambient moisture stress

C & D: Normal deposition of starch granule within the chloroplast

Plate. 21 Effect of elevated CO₂ and moisture stress on starch granules in the leaves of *Brassica juncea* cv. RH-30 (EM 8400x.)



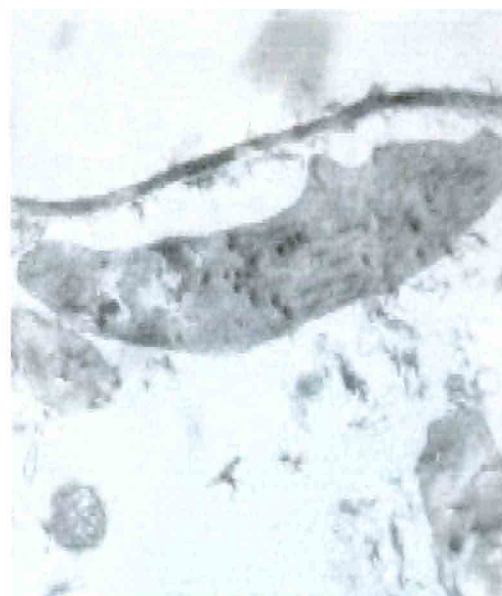
FACE irrigated



FACE moisture stress

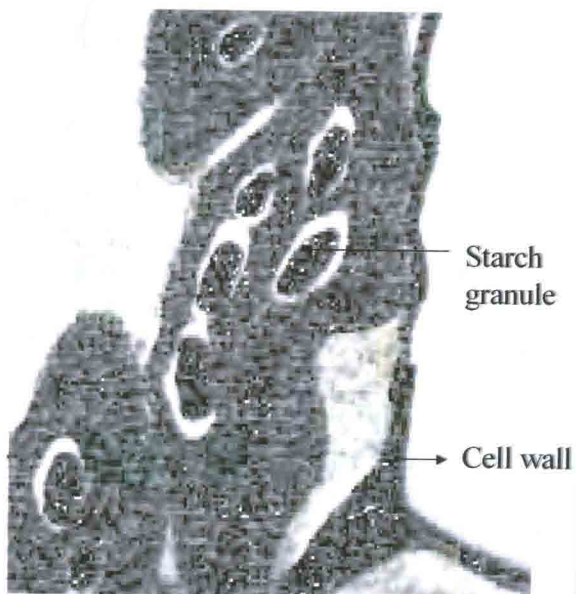


Ambient irrigated



Ambient moisture stress

Plate. 23 Effect of elevated CO₂ and moisture stress on chloroplast structure and starch granule in the leaves of *Brassica campestris* cv. Pusa Gold (EM; 8200x)



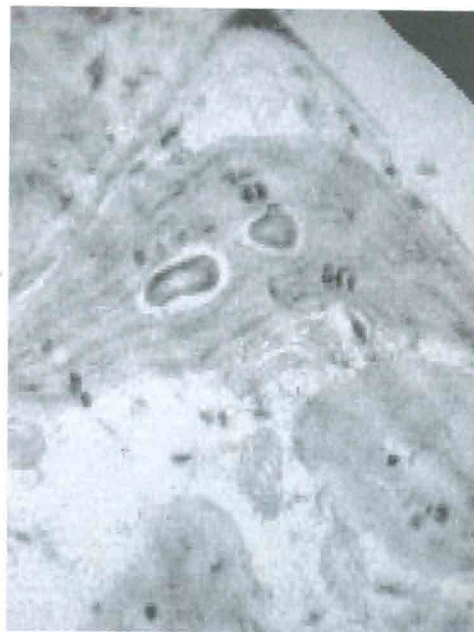
FACE irrigated



FACE moisture stress



Ambient irrigated



Ambient moisture stress

Plate. 24 Effect of elevated CO₂ and moisture stress on chloroplast structure in the leaves of *Brassica juncea* cv. RH-30. (EM; 8200x)

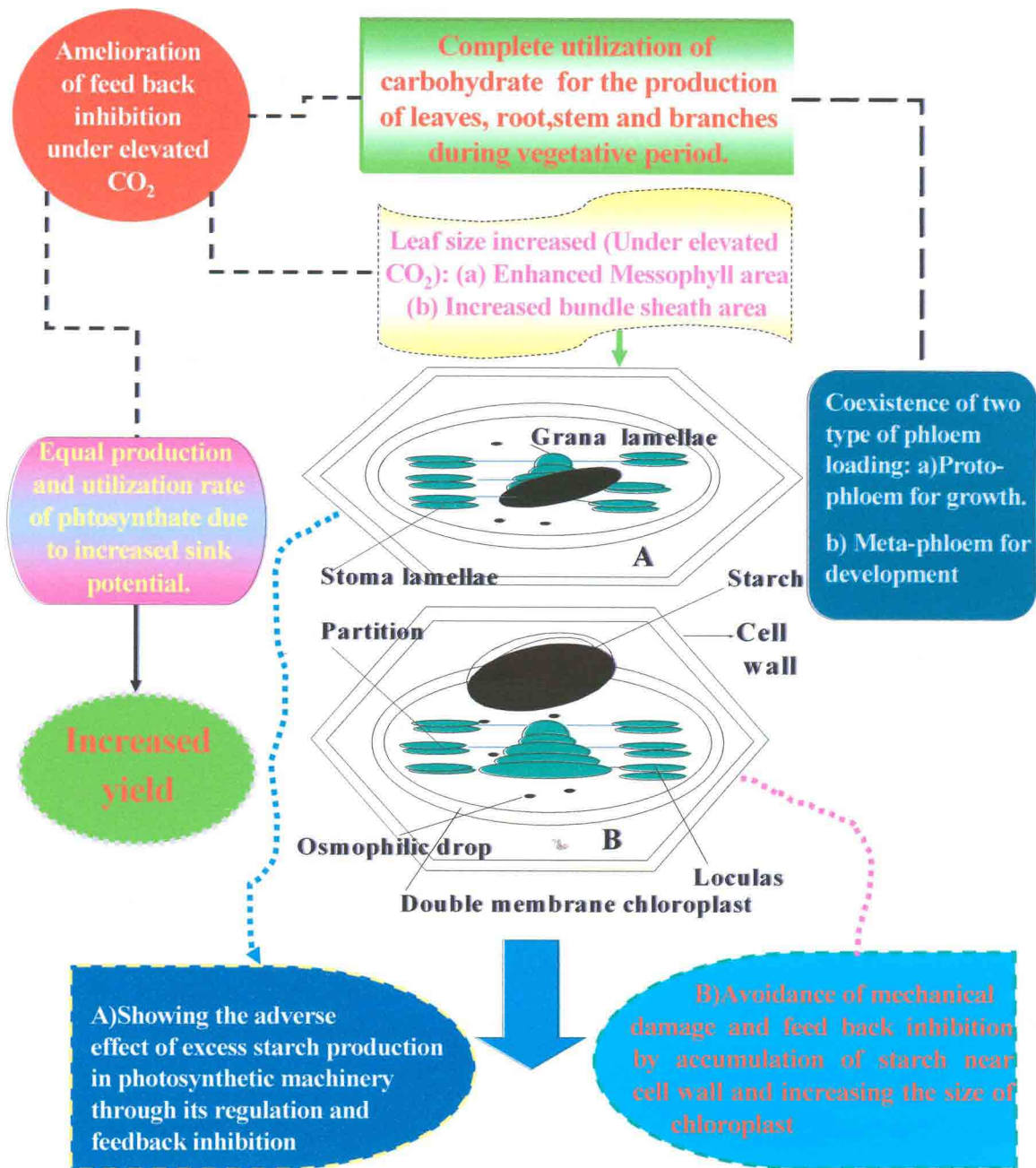


Fig. 48. Avoidance of feedback inhibition in *Brassica* cultivar under elevated CO_2 condition

and duration of these physiological responses, however, appeared to be controlled by genetic, environmental and experimental factors (Idso, 1999; Ward *et al.*, 1999 and Rogers *et al.*, 1999). Drake *et al.* (1997) in an article entitled “More efficient Plants: A consequence of rising atmospheric CO₂?” attribute it to increase in resource use efficiency by increasing carboxylation process, improving WUE promotion of root proliferation for the extraction of residual moisture or available water also added value for ameliorating the water stress effect and prolonging the physiological activities. These effects have major consequence for agriculture in a world of rising atmospheric CO₂ and climate change.

Plants growing in elevated CO₂ were able to withstand stress better and often showed a delay in the onset of stress due to lower stomatal conductance and transpiration (Allen, 1990). Another possible mechanism was enhanced osmotic adjustment by decreasing osmotic potential in plants grown at elevated CO₂ condition with the help of increased non-structural carbohydrates fructans, soluble sugars, secondary metabolite, proline etc. as observed in the present investigation. The greater size and number of starch granules additionally helped the osmotic phenomenon (Uprety *et al.*, 2002 and Vu *et al.*, 1998). Thus, maintaining higher water and turgor potential under water stress conditions.

Physiological adjustment (i.e. stomatal closure, osmotic adjustment, changes in tissue elasticity and resistance to xylem embolism) shown to be important tolerance and adaptation mechanisms that aid survival and recovery from stress. Cell wall structure and xylem vessel conduit size affected changes in tissue elasticity. The improved elastic properties of tissues indicated their superior capacity to sustain stomatal opening and water uptake under drought condition. The root structure, solute accumulation and leaf expansion may interact to determine the response of ψ_w to CO₂ enrichment.

Anatomical strategy developed by the plants under elevated CO₂ to counter drought effect showed that protophloem sieve conduct solute for structural development and metaphloem helped for the transfer of photosynthate for inducing

new sink development. Improvement in xylem structural organization helped plants to regulate the movement of residual water under stress condition and to maintain greater water status for active physiological process by delaying the onset of moisture stress.

Another mechanism that helped plants to meet the stress effect was by regulating the additional metabolic reserve for combating the oxidative stress both by inducing the detoxification mechanism and improving repair of cell membrane due to elevated CO₂.

Thus, in the present investigation the response of *Brassica* species to the interaction of elevated CO₂ and moisture stress was characterized using Free Air CO₂ Enrichment Technology. The quantitation of the effect on the photosynthetic capacity, growth response, mathematical changes in phenological parameters, biomass allocation, internal chemical changes such as accumulation of storage carbohydrates, shift in carbon nitrogen balance, modification in water relations through changes in water status and osmotic adjustment, generation of antioxidant, anatomical modification and the quality parameters, was done to generate data base to understand the strategies of plants to adjust these environmental changes at varietal level, level of stages of growth and at conditions of growth limiting environmental factors. This database on *Brassica* species will help in developing model, identification of cultivars and modification of cultivation and nutrient application technologies for future environments of higher CO₂ and moisture stress.

SUMMARY AND CONCLUSION

The anthropogenic changes, such as fossil fuel burning, deforestation, industrialization and urbanization have brought about significant rise in the atmospheric CO₂ concentration from 280 µmol mol⁻¹ CO₂ (pre-industrial period) to 370 µmol mol⁻¹ CO₂ (current level) and it is expected to be about 600 µmol mol⁻¹ CO₂ by the middle of 21st century. This rise will effectively influence the productivity of crop plants.

Innovative investigations were required to study the responses of crop plant to the elevated CO₂ and the prevailing environmental factors and the physiological and biochemical characterization of these responses. Accurate predictions of growth and developmental responses of crops to the elevated CO₂ would help farmers to select cultivars responsive to the higher concentration of CO₂.

Thus, in the present investigation the responses of two *Brassica* cultivars namely *B. campestris* cv. 'Pusa Gold' and *B. juncea*, cv. 'RH-30' to the interaction of elevated CO₂ and moisture stress, was studied, using Free Air CO₂ Enrichment (FACE) technology to simulate doubling CO₂ concentration. These *Brassica* cultivars are important oil seed varieties commercially grown for oil purposes in Northwestern region of India. They normally experience intermittent drought at various stages of growth.

Physiological and biochemical analysis of these responses was done through quantitation of the effects on the photosynthetic capacity, growth response, mathematical changes in phenological parameters, biomass allocation, internal chemical changes such as accumulation of carbohydrates, shift in carbon nitrogen balance, modification of water relations through changes in water status

and osmotic adjustment, generation of antioxidant defense system, anatomical modification and the quality parameters, to generate data base to understand the strategies of *Brassica* cultivars to adjust these environmental changes at varietal level, at different stages of growth and at conditions of growth limiting environmental factors.

Moisture stress treatment was given by restricting irrigation at different stages of growth. Observations on photosynthesis, respiration, intercellular CO₂ concentration, and stomatal conductance were recorded using infrared gas analyzer. Biochemical parameters, such as carbohydrates, antioxidative system, quality related components were studied according to standard biochemical procedures. Fatty acid composition in seeds was measured by gas liquid chromatography. Plant water relations were studied using Scholander's pressure chamber for water potential, vapor pressure osmometer for osmotic potential and Nuclear Magnetic Resonance (NMR) technology for inter cellular water movement. Anatomical studies were done by using light and transmission electron microscopy (TEM). Plants were sampled in leaf, stem and roots to measure carbon, nitrogen and dry matter accumulation at different stages of plant growth.

It was observed that the gas exchange system was stimulated under elevated CO₂ due to increase in the rate of photosynthesis in both the cultivars. The increase in photosynthesis was maximum at flowering although there was larger increase in stomatal resistance. The continuous high rate of photosynthesis throughout the growth period was attributed to the increased generation of additional foliage, reproductive organs and enhancement of stem and root growth. Elevated CO₂ with additional vegetative and reproductive growth might possibly result by the continued high rates in photosynthesis to sustain the growth of the new sinks.

The increase in photosynthesis resulted in frequent accumulation of starch, soluble sugars and non-structural carbohydrates in the leaves and other

tissues. The increased leaf biomass resulted from both the greater growth of leaf tissue and the accumulation of starch. The decreased nitrogen in these organelles was the consequence of dilution due to the greater storage of carbohydrates and shift in the size of the plant. Additional carbohydrates produced under elevated CO_2 conditions have compensated to the adverse stress effect. This was more prevalent in 'RH-30' cultivar of *Brassica juncea*.

The moisture stress treatment, which could increase the stomatal resistance and reduce the intercellular CO_2 concentration and chlorophyll content caused reduction in photosynthesis. The elevated CO_2 compensated the intercellular CO_2 depletion. Further, the decline in respiration under moisture stress condition was greater at ambient CO_2 compared to elevated CO_2 condition. This indicated the CO_2 induced compensatory mechanism of carbon balance under stress condition in *Brassica* species. The reduction in the dark respiration in *Brassica* leaves in addition to increased photosynthesis could potentially resulted in an improved carbon use efficiency and carbon balance in plants ameliorating the adverse effect of moisture stress under increased concentration of CO_2 .

Elevated CO_2 brought about significant increase in vegetative, reproductive and total crop growth period. The increase in vegetative period resulted in the complete development of CO_2 induced new branches and leaves. These newly developed leaves and branches act as temporary sinks for the photo assimilates produced due to CO_2 enrichment. Increase in reproductive period lead to the development of more number of siliquae per branch synchronizing the production of the photo assimilates. Seed filling period for both early and late initiating siliquae increased to develop high density grains and increasing the grain weight and grain yield. CO_2 enrichment not only increased the sink size but also its capacity to give additional benefit to plants to combat the adverse effect of moisture stress on yield components.

Moisture stress treatment not only reduced crop duration but also decreased the branch number, foliage and sink size but this stress-induced

reduction was ameliorated at higher level of CO₂. The greater ameliorative effect of elevated CO₂ for yield parameters was observed in 'RH-30' cultivars compared to 'Pusa gold' under moisture stress condition.

The elevated CO₂ prolonged physiological activities by maintaining the water status in plants under moisture stress condition. This was promoted by root proliferation and increased exploitation of deep soil moisture reserves, thus delaying the onset of stress. Besides the maintenance of water status, physiological adjustments such as stomatal closure, osmotic adjustment, changes in tissue elasticity and resistance to xylem embolism shown to be important tolerance and adaptive mechanisms in *Brassica* cultivars that helped in survival and recovery from stress. The increased leaf area in response to elevated CO₂ brought about an early development of full canopy, where the reduced transpiration due to decreased stomatal conductance in leaves further slowed down the soil moisture depletion. Another possible mechanism demonstrated in this study was the enhanced osmotic adjustment by accumulating non-structural carbohydrates, fructans and sugars etc. under moisture stress condition. The increased accumulation of starch, other non-structural carbohydrates, proline, free amino acids, and free fatty acids served as strong osmolites to regulate the osmotic balance in cell under stress condition. This mechanism would decrease leaf osmotic potential while maintaining both higher water and turgor potentials delaying the onset of stress. These mechanisms were more prevalent in 'RH-30' cultivar compared to 'Pusa Gold'.

Significant increase in oil content of seeds under elevated CO₂ attributed to the participation of additional CO₂ in the acetyl CoA that leads to formation of abundant malonyl CoA result in significant increase in fatty acids. It also altered the fatty acid composition, changing oil quality of seeds.

The elevated CO₂ increased the superoxide dismutase, ascorbate peroxidase, catalase and glutathione reductase activity under moisture stress and ameliorated the oxidative stress effect to the level of unstressed plant by detoxifying the

O₂⁻. Higher CO₂ brought about increase in metabolic reserves in *Brassica* plants to combat the oxidative stress by increasing its capacity for detoxification and repair. There was significantly stronger antioxidant defence system in 'RH-30' cultivar demonstrating its better protection from oxidative stress.

It was revealed that the senescence intensity in CO₂ enriched plants was significantly lower. The delayed senescence was due the decreased level of:

- (a) MDA and H₂O₂ in the leaves of CO₂ enriched plant and
- (b) The slow degradation of chlorophyll pigments due to low activity of lipoxygenase enzyme.

The delay in senescence was one of the most important consequences of increasing leaf area duration and seed filling period. Increased leaf area duration coupled with more photosynthetic rate during seed filling resulted in increased productivity of plant. Moisture stress enhanced the senescence and reduced the seed filling period but stress induced enhancement of this senescence was suppressed by elevated CO₂ and increased seed filling period. Longer seed filling period was recorded in 'RH-30' cultivar.

The anatomical study on the phloem showed that protophloem sieve imported solute for structural development and metaphloem helped for the transfer of photosynthate for inducing new sink development. Improvement of xylem structural organization helped plants to regulate maximum residual water under stress condition to maintain water status for active physiological process by delaying the onset of moisture stress.

This database on *Brassica species* has helped to understand various strategies developed by the plants due to elevated CO₂ to adjust and acclimate under moisture stress condition (Fig. 49). Results of the present study will be useful in identifying cultivars, developing model and modifying cultivation and nutrient application technologies for future environment of higher CO₂ and moisture stress.

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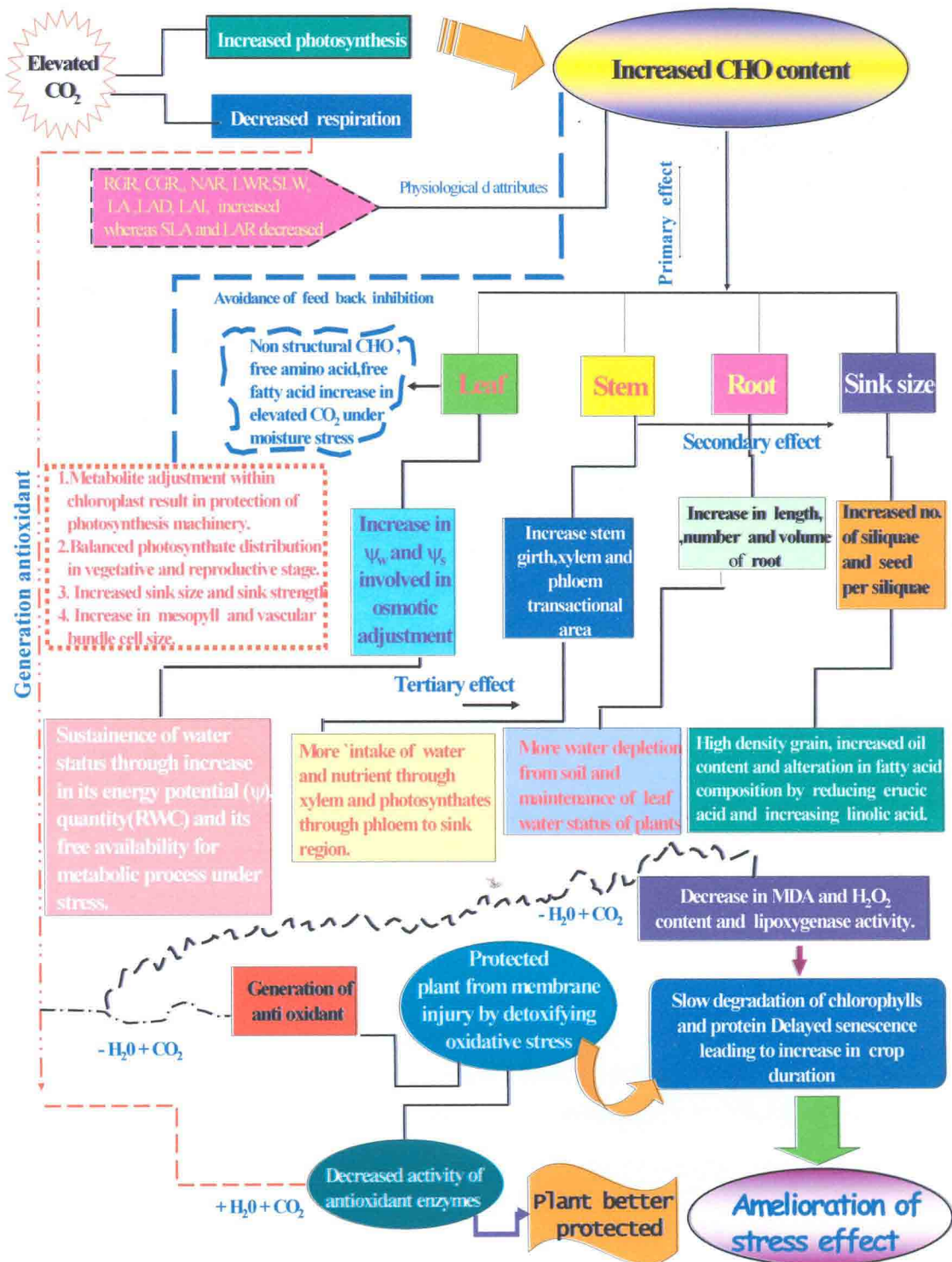


Fig. 49. Adaptation strategies induced by elevated CO₂ in *Brassica* cultivars to ameliorate the adverse effect of moisture stress.

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