Study on Growth Characteristics of Some *Brassica* Species under Moisture Stress and Elevated Carbondioxide

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**Author’s contribution**

The sole author designed, analysed, interpreted and prepared the manuscript.

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**ABSTRACT**

*Brassica juncea* and *Brassica campestris* is two important oil seed crop of North-West India experiences intermittent moisture stress during its growing period. Thus a study was carried out to ameliorate the moisture stress through elevated CO₂ applying Free Air CO₂ Enrichment (FACE) technology. The consequences of CO₂ enrichment were related to the rate of accelerated photosynthesis under both irrigated and moisture stress situation with significant decreases in stomatal conductance. The elevated CO₂ brought about a significant enhancement in all the plant growth parameters studied, and also ameliorates the of moisture stress. The carbon dioxide enrichment improves the productivity of *Brassica* cultivars viz. ‘Pusa Gold’ and ‘RH-30’ through changes in various yield attributes and also nullifying the adverse effect of moisture stress.

**Keywords:** Crop period; elevated CO₂; photosynthesis; leaf weight ratio; moisture stress; net assimilation rate; sink capacity.

1. INTRODUCTION

Of all the green house gases, 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, humidity, length of growing season, precipitation and evaporation all of which affect...
crop productivity to a great extent. The exact physiological mechanisms of rising temperatures and increased drought on yield and seed chemistry of oil seed rape are not yet resolved [1-4]. Only a few workers studied the growth response of Brassica to elevated concentrations of CO₂ [5-7] Most of our crops were selected in the past for maximum productivity at 320-330 mmol mol⁻¹ CO₂ in the atmosphere and their responses to the higher levels of CO₂ need to be characterized now for developing plant types for future [8]. This crop experiences intermittent moisture stress during vegetative to the silique formation stage, and there are reports of variability in the responses of Brassica cultivars to the moisture stress [9,10].

Among the green house gases, carbondioxide contributes a major share for global warming, and its rise in the atmosphere is likely to affect the global climate and also causes regional changes in air temperature, humidity, length of growing season, precipitation and evaporation, which affect the crop productivity to a greater extent. The exact physiological mechanisms of rising temperatures and increased drought and its effect on yield and seed chemistry of oil seed rape are not yet resolved [3,4]. Only a few workers studied the growth response of Brassica to elevated concentrations of CO₂ [6] Most of our crops were selected for maximum productivity at 320-330 mmol mol⁻¹ CO₂ in the atmosphere and their responses to the higher levels of CO₂ need to be characterized for developing better plant types for future [8]. This crop experiences intermittent moisture stress during vegetative to the silique formation stage, and there were reports on variability in the responses of Brassica cultivars to the moisture stress [9,10].

2. MATERIALS AND METHODS

2.1 Plant Materials and Experimental Design

The response of Brassica species to the elevated CO₂ was studied in the present investigation following Free Air CO₂ Enrichment (FACE) Technology to simulate the increased CO₂ concentration. Brassica cultivars viz. B. juncea cv. ‘RH-30’ and B. campestris cv. ‘Pusa Gold’ were grown in the field inside the Mid Free Air CO₂ Enrichment (mid-FACE) system in eight meters diameter circles during the winter crop season (Rabi season) at IARI, New Delhi. An elevated CO₂ concentration of 550-µ mol mol⁻¹ was maintained with the help of computer based PID valves throughout the growth period. Ambient condition was also maintained without any exogenous supply of CO₂ to the normal air under field condition.

The response of two Brassica species viz. Brassica juncea cv. RH-30 and Brassica campestris cv. Pusa gold to the elevated CO₂ were studied in the present investigation using Free Air CO₂ Enrichment (FACE) method to simulate the increased CO₂ concentration. Brassica cultivars viz. B. juncea cv. ‘RH-30’ and B. campestris cv. ‘Pusa Gold’ were grown in the field inside the Mid Free Air CO₂ Enrichment (mid-FACE) system in eight meters diameter circles during the winter crop season (Rabi season) at IARI, New Delhi. An elevated CO₂ concentration of 550-µ mol mol⁻¹ was maintained with the help of computer based PID valves throughout the growth period. Ambient condition was also maintained without any exogenous supply of CO₂ to the normal air under field condition.

2.2 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. A common computer controlled proportional integral differential (PID) valve controlled the quantity of CO₂ to be released into the arms. Depending on the downward wind direction, the central arm got CO₂ from the nozzle having 1.5 mm diameter and adjacent arms got supply from nozzles having 1.0 mm diameter. The CO₂ 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, CO₂ was always released only through 3 arms of the FACE ring/plenum. The concentration of CO₂ in open field not only depended on the quantity of CO₂ 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 PID valve controlled the quantity of CO₂ to be released into plenum arms, which depends on the control voltage, applied to it (0-10 V).
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. 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. The data from the meteorological sensors (wind speed and wind direction) along with the CO₂ concentration were measured in every second by the control system. 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.

2.3 Growth Parameters
The assimilatory area was measured using the leaf area meter (Model. LICOR 3000, USA) and leaf area index (LAI) was computed using formula suggested by Evans [11]. Leaf area duration was measured as leaf area index sustained per unit of time [12]. Height of plants were measured with the help of a meter scale from the base of plant to the apex of main axis and was recorded in centimeters. Roots were dug out at different stages of growth according to method of Sirohi et al. [13]. The length of the taproot was measured from the juncture of root and shoot to root tip by metallic measuring tape. The primary, secondary, tertiary and total number of branches per plant in each treatment was recorded at the time of harvesting the crop. Specific leaf area (SLA), specific leaf weight (SLW), leaf weight ratio (LWR), leaf area ratio (LAR) and functional growth analysis such as net assimilation rate (NAR), relative growth rate (RGR) and crop growth rate (CGR) were computed as per the method suggested by Radford [14]. Sink capacity was computed from numbers of pods per square meter, seed per pod and individual seed weight using the formula suggested by Thurling [15].

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

2.4 Gas Exchange Parameters
The CO₂ gas in the atmosphere was measured by Infra red gas analyzer in an absolute mode. Fully expanded uppermost leaf of main shoot was used for measuring the gas exchange parameters. The measurement was performed on clear sunny days between 10.00 to 12.00 hrs, when photosynthetically active radiation (PAR) ranged between 900-1500 µE m⁻² s⁻¹. The rate of photosynthesis was measured on leaves using a portable Infrared Gas Analyzer (IRGA, LI-COR-6200, Lincoln, Nebraska, USA). The stomatal conductance was measured by the method of Centritto et al. [16] using IRGA (LICOR-6200). The intercellular concentration (Cᵢ) of CO₂ was
calculated from the measured assimilation rate and stomatal conductance as follows.

\[ \frac{[g_{tc}-E/2]Ca-A}{[g_{tc}-E/2]} = g_c \]

\( g_c \) is the total conductance to CO₂.

### 2.5 Harvest Index

The harvest index was calculated as the ratio of economic yield to total biological yield and was expressed in percentage [12].

### 2.6 Yield Attributing Parameters

The yield attributing parameters viz. total number of pod per plant, number of seeds per pod, pod dry-weight per plant, seed-husk ratio and seed yield (gm²) were recorded at final harvest.

### 2.7 Statistical Analysis

Statistical analysis of data was done following the method of analysis of variance (ANOVA) given by Panse and Sukhatme [17]. The critical difference (CD) values were calculated at 5 percent probability level.

### 3. RESULTS AND DISCUSSION

#### 3.1 Number of Branch

\( \text{CO}_2 \) enrichment significantly increased the production of branches in \textit{Brassica} cultivars. The increment of primary, secondary, tertiary, and total branch was 18%, 37%, 41%, and 32% respectively (Table 1). The production of branches were significantly higher in cv. ‘RH-30’ compared to cv. ‘Pusa Gold’ cultivar. Moisture stress treatment significantly reduced the number of branches with 29% in primary, 43% in secondary, 47% in tertiary, and 30% in total number of branches under both FACE technology and ambient stress situation. The stress-induced reduction in cv. ‘Pusa Gold’ under ambient \textit{CO}_2 condition was 25% (primary), 31% (secondary), 80% (tertiary) and 34% (total branches) whereas under elevated \textit{CO}_2 condition was 19% (primary), 21% (secondary), 33% (tertiary) and 24% (total branches). On the other hand in cv. ‘RH-30’, reduction was 22% (primary), 28% (secondary), 33% (tertiary) and 31% (total branches) under ambient \textit{CO}_2 condition and it was 18% (primary), 19% (secondary), 23% (tertiary) and 22% (total branches) under elevated \textit{CO}_2 condition.

#### 3.2 Assimilatory Area (Leaf Area)

Elevated \textit{CO}_2 treatment significantly enhanced the assimilatory area (25%) throughout the growth period (Fig. 1). The response was greater in the cultivar ‘RH-30’. Moisture stress resulted in the reduction of this component up to 35%. The stress-induced reduction in assimilatory area was 28% under ambient condition whereas it was 19% under elevated \textit{CO}_2 treatment in cv. ‘Pusa Gold’. Similarly the reduction in cv. ‘RH-30’ under ambient and elevated conditions was 21% and 15% respectively.

#### Table 1. Interactive effect of elevated \textit{CO}_2 and moisture stress on different number of branches of \textit{Brassica} species

| Treatments       | Primary | Secondary | Tertiary | Total |
|------------------|---------|-----------|----------|-------|
|                  | Pusa Gold | RH-30   | Pusa Gold | RH-30 | Pusa Gold | RH-30 | Pusa Gold | RH-30 |
| FACE IRR         | 9.00    | 9.00     | 20.00    | 23.00 | 3.00     | 16.00 | 32.13     | 48.00 |
| FACE MS          | 7.30    | 7.40     | 15.60    | 18.60 | 2.00     | 12.30 | 24.30     | 37.40 |
| AMB IRR          | 8.00    | 7.60     | 16.00    | 18.80 | 2.00     | 12.90 | 26.00     | 37.50 |
| AMB MS           | 6.00    | 5.90     | 11.00    | 13.60 | 0.00     | 8.70  | 17.00     | 25.80 |
| CD= 5%           |         |          |          |       |          |       |           |       |
| CV               | 1.34    | 3.12     | 1.55     | 5.21  |
| \textit{CO}_2   | 0.59    | 1.34     | 0.45     | 2.12  |
| CV x \textit{CO}_2 | 1.12  | 1.56     | 1.21     | 3.13  |
| MS               | 0.54    | 1.12     | 0.35     | 2.78  |
| CV x MS          | 0.86    | 1.87     | 0.65     | 4.32  |
| \textit{CO}_2 x MS | 1.03  | 2.34     | 0.89     | 5.12  |
| CV x \textit{CO}_2 x MS | 1.67 | 3.99     | 1.2     | 6.98  |

VEG = Vegetative; FBI = Flower bud initiation; FL = 50% Flowering; PFL = Post flowering; FACE IRR = FACE irrigated; FACE MS = FACE Moisture stress; AMB IRR = Ambient irrigated; AMB MS = Ambient Moisture stress
Fig. 1. (a) Mid Face technology, (b) *Brassica* Crop Under Mid face
3.3 Leaf Area Duration (LAD)

CO$_2$ enrichment significantly increased (20%) the leaf area duration in *Brassica* cultivars (Fig. 2). It was significantly higher in cv. ‘RH-30’. Moisture stress caused reduction (21%) in LAD. Stress induced reduction in LAD was 35% under ambient conditions and 19% in elevated CO$_2$ condition in cv. ‘Pusa Gold’ whereas, in cv. ‘RH-30’ the reduction was 23% in ambient condition and 13% under elevated CO$_2$.

3.4 Root: Shoot Ratio

The higher-level of CO$_2$ concentration brought about marked increase in root: shoot ratio (12%). It was greater in cv. ‘Pusa gold’ compared to cv. ‘RH-30’ (Fig. 3). Moisture stress caused significant increase in root: shoot ratio (10%). The stress induced increase of root: shoot ratio was greater under elevated CO$_2$ (16% in cv. ‘Pusa Gold’ and 12% in cv. ‘RH-30’). There was no significant effect of moisture stress at ambient condition.

3.5 Net Assimilation Rate (NAR)

The CO$_2$ enrichment significantly enhanced the net assimilation rate (33%). NAR was significantly higher in cv. ‘RH-30’ compared to cv. ‘Pusa Gold’. Moisture stress significantly reduced NAR (30%) throughout the growth period. The stress-induced reduction on NAR under ambient and elevated CO$_2$ condition was 34% and 24% respectively in cv. ‘Pusa Gold’. Similarly, in cv. ‘RH-30’ the reduction was 33% and 21% under ambient and elevated CO$_2$ condition respectively (Table 3).

3.6 Leaf Area Ratio (LAR)

Elevated CO$_2$ reduced LAR in *Brassica* cultivars (23%). The LAR was lower in cv. ‘RH-30’ throughout the growth period. The interactive effect of elevated CO$_2$ and moisture stress for this character was however not significant (Table 4).

3.7 Specific Leaf Area (SLA)

There was significant reduction in SLA (20%) values under elevated CO$_2$ (Table 5). It was low in cv. ‘RH-30’. However, the interaction of CO$_2$ and moisture stress effect for this character was not significant.

3.8 Leaf Weight Ratio (LWR)

A significant increase in LWR (28%) was observed with elevated level of CO$_2$. Higher LWR was recorded in the cv. ‘RH-30’. Moisture stress brought about significant reduction (25%) in LWR. Stress induced reduction on LWR was 27% under ambient condition whereas under elevated CO$_2$ treatment it was 11% in cv. ‘Pusa Gold’. Similarly, in cv. ‘RH-30’ the reduction was 26% under ambient condition and 16% under elevated CO$_2$ condition (Table 6).

3.9 Specific Leaf Weight (SLW)

The higher level CO$_2$ brought about marked increase (35%) in SLW with high SLW in cv. ‘RH-30’. Moisture stress significantly reduced (28%) SLW. The stress-induced reduction in SLW under ambient condition was 31% whereas; under elevated CO$_2$ condition it was 13% in cv. ‘Pusa Gold’. The corresponding reduction in ‘RH-30’ was 29% and 13% respectively (Table 7).

3.10 Relative Growth Rate (RGR)

Significant increase (30%) in RGR was observed with elevated level of CO$_2$. It was higher in cv. ‘RH-30’. Moisture stress significantly reduced (37%) the RGR. Stress induced reduction for this character was 31% under ambient condition and 23% at elevated CO$_2$ in ‘Pusa Gold’, whereas; in cv. ‘RH-30’ the reduction was 26% under ambient condition and 16% under elevated CO$_2$ condition (Table 8).

3.11 Crop Growth Rate (CGR)

The pattern of CGR was recorded in (Table 9) which revealed that higher level of CO$_2$ significantly enhanced (27%) CGR in *Brassica* cultivars. It was significantly higher in cv. ‘RH-30’. Moisture stress significantly reduced the CGR (34%). Stress induced reduction was 36% under ambient condition and 21% under elevated CO$_2$ in cv. ‘Pusa Gold’. Similarly, in cv. ‘RH-30’ corresponding reductions were 27% and 19% respectively.

3.12 Photosynthesis

Elevated CO$_2$ brought about significant increase in the rate of photosynthesis in *Brassica* leaves (19%) (Fig. 4). The rate of photosynthesis was higher in cv. ‘RH-30’ irrespective of treatment. Moisture stress significantly reduce the rate of photosynthesis.
photosynthesis (20%) and reduction in photosynthesis in cv. ‘PusaGold’ under ambient and elevated conditions were 21% and 29% respectively. The stress induced reduction in photosynthesis under ambient and elevated CO$_2$ conditions were 20% and 13% respectively in cv. ‘RH-30’.

### 3.13 Yield Attributing Parameters

#### 3.13.1 Sink capacity

It was noteworthy that CO$_2$ enrichment brought about significant increase (58%) in sink capacity of *Brassica* cultivars. The cv. ‘RH-30’ had higher sink capacity (24%) compared to cv. ‘Pusa Gold’ (Table 10). Moisture stress significantly reduced sink capacity of *Brassica* cultivars (62%). The stress-induced reduction under ambient condition was 59% compared to 27% under elevated CO$_2$ condition in cv. ‘Pusa Gold’ whereas; in cv. ‘RH-30’ it was 50% at ambient condition and 24% at elevated CO$_2$ treatment.

#### 3.13.2 Harvest Index (HI)

The (Table 10) revealed that elevated CO$_2$ brought about significant increase (14%) in harvest index of *Brassica* cultivars. It was significantly higher in cv. ‘RH-30’ compared to cv. ‘Pusa Gold’. Moisture stress had no significant effect on HI. Interactive effect of moisture and CO$_2$ was also not significant.

#### 3.13.3 Seed weight (1000 seed)

The increased concentration of CO$_2$ significantly enhanced (21%) the 1000-seed weight in *Brassica* cultivars (Table 10). The 1000-seed weight was 38% higher in cv. ‘RH-30’ compared to cv. ‘Pusa gold’. Moisture stress caused significant reduction (24%) in 1000-seed weight. The stress-induced reduction on seed weight under ambient condition was 28% whereas in elevated CO$_2$ it was 11% in cv. ‘Pusa Gold’. Similarly, in the case of cv. ‘RH-30’, it was 22% under ambient and 11% under elevated conditions.

#### 3.13.4 Yield

The higher concentration of CO$_2$ significantly increases the seed yield (29%, Table 10). The cv. RH-30 registered higher seed yield irrespective of treatment. Moisture stress caused significant reduction (47%) in seed yield. The reduction in seed yield in cv. ‘Pusa Gold’ was 49% under ambient condition compared to 21% under elevated CO$_2$ condition. Similarly in case of cv. ‘RH-30’ it was 39% under ambient condition and 18% under elevated CO$_2$ condition.

The elevated CO$_2$ is expected to cause global warming and would also change the carbon balance in the biosphere by affecting the photosynthetic carbon assimilation in plants [18,19]. 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 CO$_2$ with consequence on the global food security through its effect on photosynthetic rates and thus productivity.

It was reported that C$_3$ plants (wheat, rice, oilseeds, pulses etc.) responded to elevated CO$_2$ by reducing the oxygenase activity of RuBP carboxylase oxygenase enzyme [20], changes in stomatal conductance, root growth and water use efficiency [21,22].

The present investigation revealed that the elevated CO$_2$ significantly increases the rate of photosynthesis (Fig. 4) and internal CO$_2$ concentration (Fig. 5) in both the *Brassica* cultivars. The highest photosynthetic rate, intercellular CO$_2$ concentration and decreased level of stomatal conductance were observed at flowering stage indicating the influence of elevated CO$_2$ on the sink potentiality of *Brassica* species. Because flowering is the key determinant of sink activity in this species.

Increased rate of photosynthesis and reduction in respiration rate are the two important physiological processes mostly affected by elevated CO$_2$ in crop plants [23-25]. Photosynthesis re-examined to characterize the response of *Brassica* spp. to elevated CO$_2$. It was observed that CO$_2$ enrichment brought about a marked increase in the rate of photosynthesis in both the cultivars. The increase in photosynthesis could be related to higher intercellular CO$_2$ concentration, and optimum stomatal conductance (Fig. 6). The response of leaves to an elevated CO$_2$ concentration possibly depends on the inherent sink strength of plants [26-28]. Results of our present study also exhibited increased sink strength under elevated CO$_2$ concentration. This resulted in the enhanced activities of the sources and thus photosynthetic rate. In the present investigation cv. ‘RH-30’ with its large number of leaves and siliquae and greater sink capacity responded highly to elevated CO$_2$ for photosynthesis compared to cv. ‘Pusa Gold’.
The lower rate of photosynthesis under moisture stress, which might be due to low internal CO$_2$, was greatly ameliorated by CO$_2$ 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 [29,30] attributed it partially to the depression in stomatal conductance. Sebastiani et al. [31] reported that the elevated CO$_2$ enhanced rates of net photosynthesis and decreased stomatal conductance in Oleaeuropaea and contributed it to higher water use efficiency in sorghum [32].

Plant morphological characters like branch number, leaf number; assimilatory area etc. Significantly increased due to CO$_2$ enrichment. Moisture stress induced reduction in these parameters and was greatly ameliorated by elevated CO$_2$. Excess carbohydrates produced due to CO$_2$ enrichment were 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.

Liu et al. [33] reported that the elevated CO$_2$ resulted in larger fresh mass, dry mass, leaf area and leaf thickness in two-year old needles of Sitka spruce (Piceasitchensis). Tree height, basal diameter and biomass production were also increased regardless of Nitrogen supply. Present investigation revealed that the elevated CO$_2$ brought about significant increase in root length. But Moisture stress treatment caused reduction in root length. Carbon dioxide enrichment ameliorated the stress-induced effect on these parameters.

### Table 2. Interactive effect of elevated CO$_2$ and moisture stress on leaf number at different stages of growth of Brassica species

| Treatments  | VEG | FBI | FL | PFL |
|-------------|-----|-----|----|-----|
|             | Pusa Gold | Pusa Gold | Pusa Gold | Pusa Gold |
| FACE IRR    | 0.66 | 1.12 | 1.54 | 1.46 |
| FACE MS     | 0.77 | 1.32 | 0.97 | 0.71 |
| AMB IRR     | 0.89 | 1.23 | 2.12 | 1.05 |
| AMB MS      | 1.12 | 1.78 | 2.87 | 1.88 |
| CV          | 1.77 | 2.12 | 3.67 | 2.45 |
| CO$_2$      | 0.068 | 0.087 | 0.101 | 0.054 |
| MS          | 0.078 | 0.101 | 0.110 | 0.057 |
| CV x CO$_2$ | 0.107 | 0.118 | 0.132 | 0.101 |
| CV x MS     | 0.101 | 0.132 | 0.177 | 0.086 |
| CO$_2$ x MS | 0.137 | 0.187 | 0.289 | 0.117 |
| CV x CO$_2$ x MS | 0.191 | 0.299 | 0.376 | 0.182 |

### Table 3. Interactive effect of elevated CO$_2$ and moisture stress on net assimilation rate (mg cm$^{-2}$ day$^{-1}$) at different stages of growth of Brassica species

| Treatments  | VEG | FBI | FL | PFL |
|-------------|-----|-----|----|-----|
|             | Pusa Gold | Pusa Gold | Pusa Gold | Pusa Gold |
| FACE IRR    | 0.66 | 1.12 | 1.54 | 1.46 |
| FACE MS     | 0.77 | 1.32 | 0.97 | 0.71 |
| AMB IRR     | 0.89 | 1.23 | 2.12 | 1.05 |
| AMB MS      | 1.12 | 1.78 | 2.87 | 1.88 |
| CV          | 1.77 | 2.12 | 3.67 | 2.45 |
| CO$_2$      | 0.068 | 0.087 | 0.101 | 0.054 |
| MS          | 0.078 | 0.101 | 0.110 | 0.057 |
| CV x CO$_2$ | 0.107 | 0.118 | 0.132 | 0.101 |
| CV x MS     | 0.101 | 0.132 | 0.177 | 0.086 |
| CO$_2$ x MS | 0.137 | 0.187 | 0.289 | 0.117 |
| CV x CO$_2$ x MS | 0.191 | 0.299 | 0.376 | 0.182 |
Derner et al. [34] reported that both root and shoot systems of grass seedlings responded similarly to CO₂ enrichment irrespective of whether soil water was limiting or abundant. They observed significant increase in root volume with CO₂ enrichment supporting the contention of root volume enhancement in our study (Fig.7).

The high root: shoot ratio under elevated CO₂ observed in the present studies could be attributed to longer root system with greater biomass partitioning to belowground mass as also observed by Uprety et al. [35] and Mueller [36]. Morrison [37] suggested the greater carbon allocation to roots as a mechanism to improve plant water status at elevated CO₂. The acceleration of root growth in elevated CO₂ could also result in the establishment of seedlings more rapidly and avoiding water deficit [38]. Polle et al. [39] 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 CO₂ had enhanced water uptake, improved water balance and avoided water deficit. The impact of elevated CO₂ on the WUE appeared greater under moisture stress condition.

Studies have been conducted by various worker on different crop response to elevated CO₂ and temperature and found significant change in growth parameters [40,41,42]. Elevated CO₂ brought about a significant increase in physiological attributes viz. net assimilation rate (NAR), relative growth rate (RGR) and crop growth rate (CGR) in Brassica species. The reduction in NAR, RGR and CGR was observed under moisture stress condition. However, the adverse effect on NAR, RGR and CGR was ameliorated by elevated CO₂. The present study also revealed that the elevated CO₂ concentration significantly enhanced leaf weight ratio (LWR) and specific leaf weight (SLW). On the other hand moisture stress significantly decreased the traits LWR and SLW at each stage of growth. The stress-induced intensity of reduction in the LWR and SLW was decelerated at higher CO₂ concentration. The promotion of NAR by high CO₂ contributed to elevating RGR, though high CO₂ substantially decreased LAR. High CO₂ promoted the dry matter partitioning to stem and root fraction in rice [43] and to leaf sheath stem, root and pods of Brassica species. In the present investigation in Brassica species, the increased relative growth rate due to CO₂ enrichment attributed to reduction in LAR and SLA. But increase of LWR and SLW was significant, thus promoted 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 be related to these causes. Li et al. [44] also found that when cucumber plants were in high N supply, the increase in total biomass by elevated CO₂. Yelle et al. [45] 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 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 (Table 11) possibly owing to enhanced rate of photosynthesis throughout the crop growth period. But moisture stress significantly reduced the vegetative, reproductive and total crop growth period.

Carbon dioxide enrichment improved the productivity of Brassica species 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 and harvest index. Greater response for these yield contributing characters was recorded in cv. ‘RH-30’ compared to cv. ‘Pusa gold’. Moisture stress adversely affected the yield components whereas the CO₂ enrichment brought about marked amelioration of this stress effect on yield parameters (Table 10). Reports available on the studies made on Brassica species under elevated CO₂ situation (in growth chamber) involving various temperature and moisture regimes indicated the positive influence of elevated CO₂ on growth and development and minimized the detrimental effect of adverse soil and atmospheric condition [1,46,47] Das and Uprety [48], Johannessen et al. [49,7].

It was observed that the grain weight and yield improved under CO₂ enrichment regime. This result are conformity the findings of Uddin 2018. Increase yield might be attributed to the development of numerous siliquae at frequent intervals in different branches to coincide with photoassimilate production at various newly
developed sources. This had helped in catering the demand of newly induced sinks by the excess photo assimilates produced under CO₂ enriched condition.

Table 4. Interactive effect of elevated CO₂ and moisture stress on leaf area ratio (cm² g⁻¹) at different stages of growth of Brassica species

| Treatments  | VEG Pusa Gold | FBI Pusa Gold | FL Pusa Gold | PFL Pusa Gold |
|-------------|---------------|---------------|--------------|---------------|
| FACE IRR    | 185.28        | 149.78        | 131.88       | 101.87        | 85.14         | 53.96         | 14.01         | 10.32         |
| FACE MS     | 169.49        | 139.34        | 123.93       | 96.06         | 78.36         | 52.78         | 13.24         | 9.51          |
| AMB IRR     | 212.54        | 188.14        | 165.23       | 133.74        | 117.92        | 77.34         | 20.46         | 14.42         |
| AMB MS      | 208.62        | 182.69        | 161.15       | 127.41        | 113.67        | 76.21         | 19.19         | 13.71         |
| CD = 5%     |               |               |              |               |               |               |               |
| CV          | 24.98         | 26.65         | 27.44        | 4.67          |               |               |               |
| CO₂         | 15.77         | 17.99         | 19.22        | 2.89          |               |               |               |
| CV x CO₂    | 20.21         | 22.34         | 23.67        | 3.11          |               |               |               |
| MS          | NS            | NS            | NS           | NS            |               |               |               |
| CV x MS     | NS            | NS            | NS           | NS            |               |               |               |
| CO₂ x MS    | NS            | NS            | NS           | NS            |               |               |               |
| CV x CO₂ x MS | NS         | NS            | NS           | NS            |               |               |               |

Table 5. Interactive effect of elevated CO₂ and moisture stress on specific leaf area (cm² g⁻¹) at different stages of growth of Brassica species

| Treatments  | VEG Pusa Gold | FBI Pusa Gold | FL Pusa Gold | PFL Pusa Gold |
|-------------|---------------|---------------|--------------|---------------|
| FACE IRR    | 197.21        | 155.17        | 227.14       | 170.16        | 212.18        | 157.67        | 304.25        | 215.12        |
| FACE MS     | 205.24        | 168.29        | 236.17       | 181.17        | 220.71        | 169.73        | 317.24        | 238.36        |
| AMB IRR     | 248.32        | 204.13        | 278.92       | 215.96        | 263.62        | 211.72        | 376.80        | 287.15        |
| AMB MS      | 265.42        | 236.21        | 295.20       | 231.63        | 280.31        | 192.26        | 376.80        | 287.15        |
| CD = 5%     |               |               |              |               |               |               |               |
| CV          | 50.15         | 55.45         | 57.65        | 61.22         |               |               |               |
| CO₂         | 30.34         | 32.87         | 35.89        | 40.72         |               |               |               |
| CV x CO₂    | 40.66         | 44.32         | 46.67        | 51.33         |               |               |               |
| MS          | NS            | NS            | NS           | NS            |               |               |               |
| CV x MS     | NS            | NS            | NS           | NS            |               |               |               |
| CO₂ x MS    | NS            | NS            | NS           | NS            |               |               |               |
| CV x CO₂ x MS | NS         | NS            | NS           | NS            |               |               |               |

Table 6. Interactive effect of elevated CO₂ and moisture stress on Leaf weight ratio (cm² g⁻¹) at different stages of growth of Brassica species

| Treatments  | VEG Pusa Gold | FBI Pusa Gold | FL Pusa Gold | PFL Pusa Gold |
|-------------|---------------|---------------|--------------|---------------|
| FACE IRR    | 0.16          | 0.19          | 0.20         | 0.22          | 0.23          | 0.27          | 0.09          | 0.21          |
| FACE MS     | 0.14          | 0.16          | 0.18         | 0.20          | 0.24          | 0.24          | 0.07          | 0.18          |
| AMB IRR     | 0.14          | 0.16          | 0.17         | 0.18          | 0.19          | 0.23          | 0.08          | 0.17          |
| AMB MS      | 0.10          | 0.12          | 0.12         | 0.13          | 0.12          | 0.15          | 0.06          | 0.12          |
| CD = 5%     |               |               |              |               |               |               |               |
| CV          | 0.005         | 0.004         | 0.006        | 0.006         |               |               |               |
| CO₂         | 0.006         | 0.005         | 0.007        | 0.006         |               |               |               |
| CV x CO₂    | 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         |               |               |               |
| CO₂ x MS    | 0.009         | 0.014         | 0.017        | 0.014         |               |               |               |
| CV x CO₂ x MS | 0.013       | 0.019         | 0.021        | 0.02          |               |               |               |
Table 7. Interactive effect of elevated CO\(_2\) and moisture stress on specific leaf weight (g cm\(^{-2}\)x10\(^{-3}\)) at different stages of growth of Brassica species

| Treatments    | VEG Pusa Gold | FBI Pusa Gold | FL Pusa Gold | PFL Pusa Gold |
|---------------|---------------|---------------|--------------|---------------|
|               | RH-30         | RH-30         | RH-30        | RH-30         |
| FACE IRR      | 6.80          | 8.86          | 7.69         | 9.67          |
| FACE MS       | 5.68          | 7.94          | 6.64         | 8.40          |
| AMB IRR       | 5.44          | 7.74          | 6.34         | 8.00          |
| AMB MS        | 3.70          | 5.54          | 3.96         | 5.31          |
| CD = 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         | 1.34          |
| 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          |

Table 8. Interactive effect of elevated CO\(_2\) and moisture stress on relative growth rate (g g\(^{-1}\)day\(^{-1}\)) at different stages of growth of Brassica species

| Treatments    | VEG Pusa Gold | FBI Pusa Gold | FL Pusa Gold | PFL Pusa Gold |
|---------------|---------------|---------------|--------------|---------------|
|               | RH-30         | RH-30         | RH-30        | RH-30         |
| FACE IRR      | 0.072         | 0.093         | 0.127        | 0.174         |
| FACE MS       | 0.055         | 0.075         | 0.094        | 0.132         |
| AMB IRR       | 0.059         | 0.073         | 0.095        | 0.123         |
| AMB MS        | 0.041         | 0.054         | 0.062        | 0.085         |
| CD = 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 9. Interactive effect of elevated CO\(_2\) and moisture stress on crop growth rate (gm\(^{-2}\)day\(^{-1}\)) at different stages of growth of Brassica species

| Treatments    | VEG Pusa Gold | FBI Pusa Gold | FL Pusa Gold | PFL Pusa Gold |
|---------------|---------------|---------------|--------------|---------------|
|               | RH-30         | RH-30         | RH-30        | RH-30         |
| FACE IRR      | 4.35          | 5.70          | 16.78        | 28.06         |
| FACE MS       | 3.26          | 4.24          | 12.77        | 22.21         |
| AMB IRR       | 3.73          | 4.28          | 13.20        | 22.26         |
| AMB MS        | 2.38          | 2.88          | 7.99         | 13.92         |
| CD = 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          |
Table 10. Interactive effect of elevated CO$_2$ and moisture stress on yield attributing characters

| Treatments | Seeds / Pod | 1000 grain weight (g) | Seed: husk ratio | Sink capacity (g/m$^2$) | Yield / m$^2$ | HI (%) |
|------------|-------------|------------------------|------------------|--------------------------|--------------|--------|
|            | Pusa Gold   | RH-30                  | Pusa Gold   | RH-30                  | Pusa Gold   | RH-30  | Pusa Gold   | RH-30  | Pusa Gold   | RH-30  |          |
| FACE IRR   | 42.30       | 20.30                  | 5.11         | 6.98                    | 1.23        | 1.89   | 1322.3      | 1613.0 | 728.10      | 968.3  | 49.40     | 44.3   |
| FACE MS    | 36.00       | 17.30                  | 4.53         | 6.20                    | 0.98        | 1.52   | 966.91      | 1219.9 | 572.40      | 796.1  | 39.80     | 43.9   |
| AMB IRR    | 37.30       | 17.40                  | 4.570        | 6.40                    | 1.030       | 1.68   | 1042.7      | 1229.8 | 624.20      | 872.3  | 33.80     | 40.4   |
| AMB MS     | 26.30       | 13.30                  | 3.27         | 4.99                    | 0.65        | 1.07   | 427.46      | 519.2  | 318.10      | 404.0  | 34.83     | 41.1   |
| CD= 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                    | 43.54       |        | 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       |        | 66.68       |        | NS          |        |
| CV. x CO$_2$ x MS | 1.68 |                        | 0.48         | 0.20                    | 87.02       |        | 97.13       |        | NS          |        |

Table 11. Interactive effect of elevated CO$_2$ and moisture stress on crop growth period (Days) of Brassica species

| Treatment | Vegetative | Reproductive | Seed to maturity | Seed filling period (early developed pods) | Seed filling period (Lately developed pods) |
|-----------|------------|--------------|------------------|---------------------------------------------|---------------------------------------------|
|           | 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 IRR  | 43.30      | 52.60        | 80.60       | 93.30   | 146.00      | 160.30 | 61.30      | 68.30  | 49.20      | 52.60 |
| FACE MS   | 41.60      | 50.60        | 76.30       | 85.30   | 133.30      | 151.60 | 56.60      | 63.60  | 45.60      | 53.00 |
| AMB IRR   | 41.30      | 51.30        | 76.00       | 86.30   | 134.30      | 151.30 | 56.50      | 63.30  | 46.00      | 40.00 |
| AMB MS    | 33.60      | 43.00        | 66.00       | 76.30   | 117.00      | 132.00 | 43.00      | 49.00  | 35.00      | 56.30 |
| CD= 5%    |            |              |             |         |             |       |            |        |            |       |
| cv.       | 1.555      |              | 9.869       |        | 10.977      |        | 4.922      |        | 1.514      |       |
| CO$_2$    | 2.423      |              | 5.001       |        | 5.387       |        | 2.736      |        | 3.292      |       |
| CV. x CO$_2$ | 2.550   |              | 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      |       |
Fig. 1. Leaf area MS = moisture stress and IRR = irrigated

Fig. 2. Leaf area duration

Fig. 3. Root: shoot ratio

Fig. 4. Photosynthesis
Fig. 5. Internal CO₂ concentration

Fig. 6. Stomatal conductance (SC)

Fig. 7. Root volume (cc)

Fig. Effect of elevated CO₂ and moisture stress on various physiological parameters of Brassica species
4. CONCLUSION

There was variability in cultivar in terms of growth and yield response under higher level of CO$_2$ and moisture stress condition. The Moisture stress was significantly decreased yield attributing character but increased levels CO$_2$ (550 ppm) to be ameliorating its negative effect. *Brassica juncea* cv. RH-30 was responding more positively under elevated CO$_2$ and moisture stress condition and gave the better yield compared to *Brassica campestris* cv. Pusa Gold due to their some adaptive characters like maintenance of photosynthetic rate and other growth related attributes like NAR, RGR, CGR, root volume, root: shoot ratio. So, cv.RH-30 could be utilized in changing climatic condition for sustainable productivity. it would also be used as breeding material for development of stress resistant variety in near future.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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