Interactive Effect of Elevated CO$_2$ and Drought Stress on Leaf Anatomy in Brassica Species

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**Author’s contribution**

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

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**ABSTRACT**

The aim of present study was to understand how the ultrastructure of the leaf mesophyll cells in Brassica leaf can be altered under elevated CO$_2$ by interactive effect of elevated CO$_2$ on leaf anatomy and ultra structure of Brassica species under moisture stress conditions. Results of the experiment revealed that the crop genotypes differ greatly in response to elevated CO$_2$ and moisture stress conditions. Elevated CO$_2$ brought about an increase in cell and chloroplast expansion in Brassica genotypes. Elevated CO$_2$ also increased the thickness of epidermis, size of mesophyll cells, accumulation of starch and size and number of starch granules per chloroplast in Brassica juncea and Brassica juncea cultivars. These alterations in the ultra structure of cells in plants might help to plant adjustment to changing climate in the future.

**Keywords:** Elevated CO$_2$; moisture stress; chloroplast; starch granule and leaf thickness.

**1. INTRODUCTION**

The CO$_2$ concentration in the atmosphere was in a steady state at 280 mol mol$^{-1}$ till the pre-industrial period (1850). If it continues at the present rate i.e.1.5 ppm (exponential rise of CO$_2$ in the atmosphere); it may be doubled by the end of 21$^{st}$ century (Luo and Moony 1996). Atmospheric CO$_2$ increased to 403.3 ± 0.1 μmol mol$^{-1}$ in 2016, approximately 145% of pre-industrial level [1]. This increase will inevitably continue [2]. CO$_2$ has biological effect as well,
because it serves as the main substrate for photosynthetic carbon assimilation. However, there is no consensus on the quantitative effects of increased CO$_2$ 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. Hence, the various researches are being carried on to study the crop response to elevated CO$_2$. The current increase in atmospheric CO$_2$ at the rate of 1.5-1.8 µmol mol$^{-1}$ per annum is much too small a signal for field experiment to detect a response. Therefore, various models were used for CO$_2$ experiments using Open Top Chambers (OTCs) and Free Air CO$_2$ Enrichment (FACE) technologies that provide ample input signal or pulse which allows tracing of crop responses for a short time frame. Crops responds directly to rising CO$_2$ through photo-synthesis and stomatal conductance [3,4], which consequently promotes crop yield [5,6]. The direct fertilization effect of rising atmospheric CO$_2$ is expected to offset the reduction of crop yields induced by climate change [7]. Besides the physiological and biochemical process elevated CO$_2$ has also effect on anatomical processes depending on species. Roger et al. [8] reported in increasing 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 [9]. Leadley [10], Wu et al. [11] reported that 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 [12] 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. Chloroplasts of CO$_2$ enriched leaf tissues of plants were more deeply stained by crystal violet and appear eddenser. Elevated CO$_2$ stimulated cell production in roots cells [13]. Elevated CO$_2$ brought about an increase in cell and chloroplast expansion of wheat (Triticum aestivum) leaves 10 and 25%, respectively and observed that older leaves chloroplast contained larger starch deposits [14]. Similarly, Hao et al. [15], reported that elevated CO$_2$ increased the number and size of starch grains in chloroplasts of two cultivars of soybean. In general, simultaneous exposure to increased O$_3$ reduced the impact of increased CO$_2$ [16]. 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 [17].

Changes in chloroplast density or volume which occur in plants grown in CO$_2$ enriched environment have been generally attributed to increased starch accumulation in the chloroplasts of tomato and soybean [18]. In view of the wide ranges of results that have been obtained through previous research further studies are in the need to understand its ramifications and connection with ongoing rise in the ambient CO$_2$ content particularly in timing and mechanism of plant adjustment to elevated CO$_2$ through anatomical modification which will significantly influence in the biomass production potential or productivity of crop Brassica species viz. Brassica juncea cv. ‘RH-30’ and Brassica campestris cv. ‘Pusa Gold’ under elevated CO$_2$ and moisture stress condition.

2. MATERIALS AND METHODS

2.1 Plant Material

Brassica cultivars viz. Brassica juncea cv. RH-30 and Brassica campestris cv. Pusa Gold were collected and grown for the present investigation.

2.2 Experimental Site and Growth Conditions

The response of both the species to elevated CO$_2$ was studied using Free Air CO$_2$ Enrichment Technology (FACE) to simulate the doubling CO$_2$ concentration at, IARI, New Delhi-12. The crops were grown in the field and inside the Mid Free Air CO$_2$ enrichment (FACE) facility in 8 m diameter circles. An elevated CO$_2$ concentration of 550 µmol mol$^{-1}$ was maintained throughout the crop growth period with the help of computer-based PID valves. There was no exogenous supply of CO$_2$ to the normal air under ambient field condition. Field was prepared by recommended agronomic practices.

2.3 Cultural Practice

Farm yard manure was applied at the rate of 5 tons per hectare at the time of field preparation. The plant spacing, fertilizer application at the rate of 30+30:60:40 kg per hectare of nitrogen, phosphorus and potassium and other cultural practices were followed as reported by Uprety et al. [17].
2.4 Moisture Stress Treatment

Moisture stress treatment was given by restricting irrigation and bringing the soil moisture level between 7 and 10% compared to 22-25% under irrigated condition. All the observations were taken in triplicate for each treatment at Stage-1: vegetative (25 days after sowing), Stage-2: Flower bud initiation (45 DAS), Stage-3: 50% flowering (60 DAS) and Stage-4: post flowering (75DAS).

2.5 Leafanatomy

For the measurement of leaf thickness, leaf segment of each treatment was initially placed in fixative FFA, comprising of formalin: acetic acid: ethyl alcohol: Water (10: 5: 50: 35V/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 safranine (0.5%, w/v) in 50 %,(v/v)alcohol. The thickness of palisade, mesophyll and combined epidermal layer were measured in terms of micrometer (μm) [19].

2.6 Leaf Ultra structure Study

Ultra structural measurements were done on fully expanded leaf (6th node from the top) of mainstream using Transverse Electron Microscope (TEM) technology as reported by Robertson and Leech [20]. From the base of the leaf, slices of 2-3 mm thickness were cut and fixed in 2.5% glutaraldehyde (in 0.1 M phosphate buffer, pH7.2) at room temperature for 1 hr.

The leaf tissues were washed three times in 0.1 M phosphate buffer, pH 7.2, before post-fixation in 1 percent (v/v) osmium tetro oxide 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.) [21]. 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.

3. RESULTS

3.1 Anatomical Characters Leaf Thickness

Elevated level of CO₂ brought about significant increase in leaf thickness (Plate 1, Table 1). The increased over the ambient plant was 39% (palisade), 34%(spongy), 36%(mesophyll), 29% (upper epidermis), 28 % (lower epidermis) and 34% (total leaf thickness).Moisture stress treatment significantly reduced the leaf thickness. The reduction was 22% (palisade), 19% (spongy), 18% (mesophyll), 16% (upper epidermis), 25% (lower epidermis) and 18 (total leaf thickness) compared to control. The stress induced reduction in Pusa gold under ambient condition 39% (palisade),37%(spongy),38% (mesophyll), 31% (upper epidermis),35% (lower epidermis), 37% (total thickness), where as reduction under elevated CO₂ condition was 24% (palisade),21% (spongy), 23% (mesophyll), 22% (upper epidermis),21% (lower epidermis), 22% (total leaf thickness). The stress induced reduction in RH-30 under ambient condition 35% (palisade), 33% (spongy), 34% (mesophyll), 25% (upper epidermis), 25% (lower epidermis) and 35% total leaf thickness where as reduction in elevated CO₂ condition 21% (palisade), 17% (spongy), 19% (mesophyll), 17% (upper epidermis), 16% (lower epidermis) and 19% (total leaf thickness).

3.2 Leaf Cell Size Palisade Cells Size

The increased concentration of CO₂ significantly enhanced the palisade cell size in Brassica cultivars Table 2, Plate 2 The increase was 36% (length), 23% (breath) and 65%(area). The larger palisade cell was observed in leaves of RH-30 compared to Pusa gold. Moisture stress treatment significantly reduced cell size. The reduction was 38% (length) and 28% (breath) and 74%(area). The stress-induced reduction in Pusa gold under ambient condition was 35% length, 28% breath, and 53% area whereas reduction under elevated CO₂ was 22% (length), 16% (breath), and 35% (area). The stress-induced reduction in RH-30 under ambient condition was 28% length, 23% breath and 53% area where as in elevated CO₂ reduction was 19% (length),12%(breath) and 30% (area).

3.3 Spongy Cell Size

There was no any significant effect on spongy cell size in leaves of Brassica cultivars due to CO₂ enrichment (Table 3). The larger spongy cell was observed in leaves of Pusa gold compared toRH-30. Moisture stress treatment significantly reduced spongy cell size. The reduction was 12% (length), 12%(breath) and 21% (area). The stress-induced reduction in Pusa gold under ambient condition was 12% (length), 16% (breath) and 26% (area) however
in RH-30 was 17% (length), 17% (breath) and 19% (area).

### 3.4 Palisade Number

The increased concentration of CO₂ significantly enhanced (12.33%) the palisade cell number in *Brassica* cultivars. (Table 2). The more number of palisade cell was observed in leaves of RH-30 compared to Pusa gold. Moisture stress treatment significantly reduced (31%) number of palisade cell. The stress induced reduction in Pusa gold under ambient was 31% where it was 21% in elevated CO₂ condition. The reduction in RH-30 under ambient was 24% where as 17% in under elevated CO₂ condition.

### 3.5 Spongy Cell Number

There was no any effect on spongy cell number due to CO₂ enrichment (Table 3). The more number of spongy cells was observed in leaves of Pusa gold compared to RH-30. Moisture stress treatment significantly reduced (14%) number of spongy. The stress reduction in Pusa gold under ambient was 20.00% where as in RH-30 it was 15%.

### 3.6 Chloroplast Ultra-structure

#### 3.6.1 Mesophyll chloroplast number

The CO₂ enrichment brought about significant increase (72 %) in the chloroplast numberin the mesophyll. (Table 4) The more number of chloroplast was 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 was 34% whereas under elevated 22% in Pusa gold. The reduction in RH-30 under ambient was 32%, where as in elevated 19%.

#### 3.6.2 Vascular sheath chloroplast number

The CO₂ enrichment significantly enhanced (36%) the chloroplast number in vasculars heath cell. There was no significant difference between cultivars. Moisture stress significantly decreased (25%) in the chloroplast number in vascular bundle cell. The stress induced reduction in chloroplast number under ambient was 34% whereas in elevated condition, 21% in Pusa gold. The reduction in RH-30 under ambient was 33%, where elevated CO₂ condition only 17%.

#### 3.6.3 Number of starch granule in mesophyll chloroplast

Elevated CO₂ brought about significant increase (35%) in the number of starch granule in the mesophyll chloroplast. Moisture stress significantly decreased (33%) the number of starch granule in the mesophyll chloroplast. The stress induced reduction in number of starch granule in chloroplast under ambient was 48% where in elevated 24% in Pusa gold. The reduction in RH-30 under ambient was 46%, where elevated 21%.

#### 3.6.4 Number of starch granule in vascular sheath chloroplast

The CO₂ enrichment significantly increased (41%) the starch granule in the vascular sheath chloroplast (Table 4). Moisture stress significantly decreased (19%) the number of starch granule in the vascular sheath chloroplast. The stress induced reduction in number of starch granule in chloroplast under ambient was 42% where as elevated condition 20% in Pusa gold. The reduction in RH-30 under ambient was 33%, where elevated condition 15%.

### 4. DISCUSSION

Changes in rate of cell division, cell expansion and cell cycling was reported by various scientist due to exposure to elevated CO₂ and that might alter plant structure for example an alteration in plant structure under elevated CO₂ at organ level is possibly a result of metabolic changes induced at cellular level [22]. Elevated CO₂ brought about an increase in leaf thickness, which was also more prevalent in ‘RH-30’, whereas stress-induced reduction in leaf thickness greatly ameliorated Plate 1. The increase of the palisade and spongy layers alongwith that of epidermal cell layers contributed to the total leaf thickness under elevated CO₂ though increased CO₂ concentration and has no significant effect on cell size and number inspongy cells but a significant increase in palisade cell size was recorded and this results were in accordance with the results of Zheng et al. [23] reported in soybean which showed spongy tissue area of 15 and 28% as CO₂ concentration increased from 400 ppm to 600 ppm. Moisture stress significantly decreased the cell size and number in spongy layer. The increase in epidermal layer was one of most important strategy of plant for
Table 1. Interactive effect of elevated CO₂ and drought stress on leaf anatomy *Brassica Species*

| Treatments    | Palisade (µm) | Spongy (µm) | Upper Mesophyll (µm) | Lower Epidermis (µm) | Total (µm) | Palisade (µm) | Spongy (µm) | Mesophyll (µm) | Upper Epidermis (µm) | Lower Epidermis (µm) | Total (µm) |
|---------------|---------------|-------------|----------------------|----------------------|------------|---------------|-------------|----------------|----------------------|----------------------|------------|
| FACEIRR       | 88.19         | 99.90       | 188.10               | 27.21                | 230.71     | 73.89         | 84.21       | 158.10         | 23.20                | 15.30                | 196.60     |
| FACEEMS       | 66.34         | 78.40       | 144.80               | 21.20                | 181.20     | 57.89         | 69.12       | 127.01         | 19.10                | 12.70                | 158.81     |
| AMBIRR        | 70.23         | 80.40       | 150.60               | 22.20                | 188.90     | 57.94         | 70.14       | 128.08         | 19.20                | 12.80                | 160.08     |
| AMBMS         | 42.21         | 50.21       | 92.40                | 15.10                | 117.90     | 37.21         | 46.90       | 84.11          | 14.30                | 9.6                   | 108.01     |

FACE = Free air CO₂ enrichment, IRR= Irrigated, MS= moisture stress, AMB= ambient

Table 2. Interactive effect of elevated CO₂ and moisture stress in leaf palisade cell number and cell size

| Treatment    | Number | Pusa gold | RH-30 |
|--------------|--------|-----------|-------|
|               | Length(µm) | Breath(µm) | Area(µm²) | Length(µm) | Breath(µm) | Area(µm²) |
| FACEIRR       | 23.00    | 37.40     | 22.60 | 845.24 | 27.00 | 48.50 | 1256.15 |
| FACEEMS       | 18.00    | 28.90     | 18.81 | 543.61 | 22.30 | 38.90 | 879.14 |
| AMBIRR        | 19.00    | 29.20     | 19.70 | 575.24 | 21.00 | 39.12 | 891.94 |
| AMB MS        | 13.00    | 18.90     | 14.10 | 266.49 | 15.90 | 28.0  | 487.20 |
| CV            | 1.01     | 1.67      | 1.93  | 37.96 | 1.01 | 1.67 | 37.96 |
| CO2           | 1.58     | 2.22      | 2.45  | 81.35 | 1.58 | 2.22 | 81.35 |
| CVxCO2        | 2.56     | 3.78      | 3.47  | 115.04 | 2.56 | 3.78 | 115.04 |
| MS            | 1.67     | 2.92      | 2.43  | 76.90 | 1.67 | 2.92 | 76.90 |
| CVxMS         | 2.87     | 4.01      | 3.44  | 108.75 | 2.87 | 4.01 | 108.75 |
| CO2xMS        | 3.54     | 5.85      | 3.69  | 118.75 | 3.54 | 5.85 | 118.75 |
| CVxCO2xMS     | 4.09     | 7.44      | 4.86  | 153.80 | 4.09 | 7.44 | 153.80 |
Table 3. Interactive effect of elevated CO$_2$ and moisture stress leaf spongy cell number and cellsize

| Treatment       | Number | Length(µm) | Breath(µm) | Area(µm$^2$) | Number | Length(µm) | Breath(µm) | Area(µm$^2$) |
|-----------------|--------|------------|------------|--------------|--------|------------|------------|--------------|
| FACEIRR         | 17.00  | 31.20      | 22.90      | 714.48       | 14.00  | 26.60      | 19.44      | 517.1        |
| FACE MS         | 16.00  | 30.64      | 21.42      | 650.18       | 13.00  | 24.97      | 18.91      | 472.18       |
| AMBIRR          | 18.00  | 32.62      | 24.30      | 792.67       | 15.00  | 28.22      | 22.46      | 533.64       |
| AMBMS           | 15.00  | 28.62      | 20.30      | 580.98       | 13.00  | 23.42      | 18.44      | 431.86       |
| 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           | NS     | 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        |

Table 4. Interactive effect of elevated CO$_2$ and drought stress ultra structure of chloroplast

| Treatments       | Number of chloroplast in mesophyll | Number of starch granule in mesophyll | Number of chloroplast in Vascular sheath | Number of starch granule in vascular sheath |
|------------------|-----------------------------------|---------------------------------------|------------------------------------------|--------------------------------------------|
|                  | Pusa gold | RH-30 | Pusa gold | RH-30 | Pusa gold | RH-30 | Pusa gold | RH-30 | Pusa gold | RH-30 |
| FACEIRR          | 7.50      | 8.90  | 3.70      | 3.90  | 3.20      | 3.50  | 3.00      | 3.20  |
| FACE MS          | 5.80      | 7.20  | 2.80      | 3.10  | 2.50      | 2.90  | 2.40      | 2.70  |
| AMBIRR           | 4.70      | 5.50  | 2.90      | 2.90  | 2.60      | 2.70  | 1.90      | 1.80  |
| AMBMS            | 3.10      | 3.70  | 1.50      | 1.50  | 1.70      | 1.80  | 1.10      | 1.20  |
| CV               | 84        | NS    | 0.08      | 0.09  | 0.12      | 0.12  | 0.06      | 0.05  |
| CO$_2$           | 11        | 0.11  | 0.12      | 0.12  | 0.11      | 0.12  | 0.30      | 0.21  |
| Var x CO$_2$     | 43        | 0.43  | 0.43      | 0.43  | 0.42      | 0.42  | 0.30      | 0.30  |

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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. Uprety et al. [17] also reported at elevated CO₂ concentration has significant effect on leaf structure of Brassica juncea. Line Bio-141(95) under moisture stress which revealed that CO₂ elevated to 600 μmolmol⁻¹ increased the length of epidermal cell and length of palisade parenchyma cells Ferris and Taylor (1999) in Populus demonstrated that carbon induced growth effect occurred because of the enhanced wall extensibility and the effect of wall loosening enzyme, xyloglucan endo transglycosylase (XET). Masale [24] and Zhuo et al. [13] observed increased supply of soluble carbohydrates stimulated both cell wall expansion and cell production but the mechanism remained elusive. Ferris et al. [25] reported that leaf expansion was due to the production of larger cells in their in P. nigra when they raise the atmospheric CO₂ under FACE experiment. According to Thomas and Harvey [26] differences in thickness were due to the result of increased palisade at initiation and development. According to them in soybean and sweet gum at elevated CO₂ due to higher carbon accessibility increased osmotic potential in the leaves that in turn causes expansion of cells of palisade area. Elevated CO₂ induced one more layer of palisade cells in the mesophyll and greater cell expansion in Brassica leaves in the present investigation.

Vaz et al. [27] reported that when leaves of Quercus cussubera was exposed to elevated CO₂ it resulted in increased leaf thickness and an additional layer of palisade cells was also observed.

Uprety 2001 opined that increase in mesophyll layer served as an important approach for Brassica juncea to provide room for higher starch accumulation under high CO₂. In the present study, with Brassica, the length and width of epidermal cells were increased at high CO₂ condition, indicating greater effect on cell expansion, which might be associated with an increase in osmotic potential due to accumulation of saccharides. These changes along with those in stomatal characters might help in regulating and reducing transpiration, gas exchange and also regulating osmotic movement of cell sap to maintain turgor under moisture stress condition. Increased chloroplast size has been a reliable observation under elevated CO₂ enrichment as reported by some workers [28,17]. According to them this has been mostly attributed to increased cross-sectional width of chloroplast rather than length. The CO₂ enrichment significantly increased the chloroplast number in both mesophyll and vascular bundle sheath of the cell in Brassica leaves but it markedly decreased under 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 and laying near the cell wall under elevated CO₂ (Plate 2). The increased chloroplast number could be related with increase in the cell size. These findings indicated that cells have got more space to develop number of chloroplast. Similarly CO₂ enrichment brought about significant increase in the starch granule number within the chloroplast both mesophyll and vascular bundle sheath cell. There was no significant difference in terms of cultivars. Moister stress decreased the starch granule number within chloroplast in both mesophyll and vascular bundle sheath cells (Plates 3 and 6).

Several workers have studied the response of higher level of atmospheric CO₂ on starch accumulation in the leaves of various plants for example; number and size of starch increased in cucumber [29]; in Gmelina arborea [30] and in Isatis indigotica [15] grown under Free air atmospheric CO₂ enrichment facility. And same trend was also recorded [31] in the leaves of Impatiens hawker when treated with higher level of CO₂ in growth chamber.

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 [17]. 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 thylakoids membrane. The important phenomenon observed in the present study showed that CO₂ enrichment not only increased chloroplast size but also strengthened the chloroplast membrane. The elevated CO₂ increased the starch granule size and it
deposited them to near chloroplast membrane stretching without any damage [Plate 4.(a) & (b)]. Due to deposition of starch granule near the membrane the thylakoid and grana were protected from mechanical damages in the middle portion of chloroplast and ultimately avoid the feedback inhibition by metabolite adjustment Plate 5. The greater size and number of starch granules additionally helped the osmotic phenomenon [32,33].

Plate 1. Effect of elevated CO2 and moisture stress on leaf anatomy of *B. Campestris* cv Pusa gold and *B. juncea*, cv. RH-30 (LM100x)

Plate 2. Effect of elevated CO2 and moisture stress on vascular bundle cell size and number of chloroplast in the leaves *Brassica campestris* cv Pusa gold *Brassica juncea* cv. RH-30
Plate 3. Effect of elevated CO2 and moisture stress on chloroplast structure in the leaves of *Brassica juncea* cv. RH-30. (EM;8200x)

Plate 4(a). Effect of elevated CO2 and moisture stress on starch granules in the leaves of *Brassica juncea* cv.RH-30(EM 8400x.)

Plate 4(b). Effect of elevated CO2 and moisture stress on starch granule in the leaves of *Brassica campestris* cv. Pusa Gold (EM 8400x)
5. CONCLUSION

To understand the mechanism of responses to elevated CO$_2$, an alteration in anatomical and leaf ultra-structure is very much pertinent which provides the pave for an idea about adaptation strategies of plant under future environment. Thus, in the present investigation the response of *Brassica* species to the interaction of elevated CO$_2$ and moisture stress was characterized using Free Air CO$_2$ Enrichment technology.

Among the two cultivar cv. RH-30 showed a better adaptation by optimizing various ultra-structure such as number starch granule in chloroplast, leaf thickness, cell size etc. compared to cv. Pusa gold This data base on *Brassica* species might help in developing model, Identification of cultivars and modification of cultivation and nutrient application technologies for future environments.

**DISCLAIMER**

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

**COMPETING INTERESTS**

Author has declared that no competing interests exist.

**REFERENCES**

1. WMO. Greenhouse Gas Bulletin. The State of Greenhouse in the Atmosphere Based on Global Observations Through, 2016. 2017;1330.

2. IPCC (Intergovernmental Panel on Climate Change) Climate change. Synthesis report. Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change, the core writing team, Pachauri RK, Meyer LA. Geneva: IPCC. 2014;40–54.
3. Ainsworth EA, Rogers A, The response of photosynthesis and stomatal conductance to rising [CO$_2$]: Mechanisms and environmental interactions. Plant Cell Environ. 2007;30:258–270. Available:https://doi.org/10.1111/j.1365-3040.2007.01641.x.

4. Franks PJ, Adams MA, Amthor JS, Barbour MM, Berry JA, Ellsworth DS, Farquhar GD, Ghanoum O, Lloyd J, McDowell N, Norby RJ, Tissue DT, von Caemmerer S. Sensitivity of plants to changing atmospheric CO$_2$ concentration: From the geological past to the next century. New Phytol. 2013;197:1077–1094.

5. Long SP, Ainsworth EA, Leakey ADB, Nosberger J, Ort DR. Food for thought: Lower-than-expected crop yield stimulation with rising CO$_2$ concentrations. Science 2006;312:1918–1921.https://doi.org/10.1126/science.1114722.

6. Ainsworth, E.A., 2008. Rice production in a changing climate: a meta-analysis of responses to elevated carbon dioxide and elevated ozone concentration. Glob. Change Biol. 14,1642–1650. Available:https://doi.org/10.1111/j.1365-2486.2008.01594.x.

7. Müller C, Bonneau A, Popp A, Waha K, Fader M. Climate change impacts on agricultural yields. (Background note to the World Development Report 2010). The World Bank, Washington DC. 2010;1–11.

8. Rogers HH, Thomas JF, Bingham G. Response of agronomic and forest species to elevated atmospheric carbon dioxide. Sci. 1983;220:428–429.

9. Jarvis PG. Specific leaf weight equals 1.0 always! (Letter to editor) Hort. Sci. 20: 812. Jensen,W.A. 1962. Botanical Histochemistry. Freedom and Company San Francisco,USA;1985.

10. Leadley PW, Reynolds JA, Thomas JF, Reynolds JF. Effects of CO$_2$ enrichment of internal leaf surface area in soybeans. Bot.Gaz.1987;148:137-140.

11. Wu X-J, Sun S, Xing G-M, Wang G-L, Wang F, Xu Z-S, Tian Y-S, Hou X-L, Xiong A-S. Elevated Carbon Dioxide Altered Morphological and Anatomical Characteristics, Ascorbic Acid Accumulation and Related Gene Expression during Taproot Development in Carrots. Front.PlantSci. 2017;7:2026. DOI:10.3389/fpls.2016.02026

12. Overdieck D, Ungemach E. Effects of atmospheric CO$_2$ enrichment on the leaf anatomy of white clover (Trifolium repens L.). Verhandlungen-Gesellschaft-fur-Okologie. 1989;18:431-436.

13. Zhuo X, Misaghi IJ, Hawes MC. Stimulation of border cell production in response to increased bondioxide level. Plant Physiol. 2000;122:181-188.

14. Robertson EJ, Leech RM. Significant changes in cell and chloroplast development in young wheat leaves (Triticumaestivumcv. Hereward) growth in elevated CO$_2$. Plant Physiol. 1995a;107:63-71.

15. Hao X, Li P, Feng Y, Han X, Gao J, Lin E and Han Y. Effects of fully open-air [CO$_2$] elevation on leaf photosynthesis and ultrastructure of Isatisindigotica Fort. PLoS One. 2013;8:1–7.

16. Utirainen J, Holopainen T, Paoletti E. Ultrastructural and growth responses of young scots pine seedlings (Pinuslyvestris) on increasing carbon dioxide and ozone concentrations. Special issue: Stress factors and air pollution. Selected papers from the 17th International meeting for specialists in air pollution effects on forest ecosystem shed in Florence, Italy,14-19 September 1996.Chemosphere.1998;36:795-800.

17. Uprety D, Dwivedi N, Mohan R. Effect of Elevated CO$_2$ Concentration on Leaf Structure of Brassica Juncea under Water Stress. Biologia Plantarum. 2001;44:149–152. Available:https://doi.org/10.1023/A:1017959429783.

18. Madsen E. Cytological changes due to the effect of carbondioxide concentration on the accumulation of starch in chloroplasts of tomato leaves.Royal Veterinary and Agricultural University, Copenhagen. Year book.1971;191-194.

19. Rahim MA, Fordham RR. Effect of Shade on leaf and cell size and number of epidermal cells ingarlic (AlliumsativumL..) Ann.Bot. 1991;67:167-171.

20. Robertson EJ, Williams M, Harwood JL, Lindsay JG, Leaver CJ, Leech RM. Mitochondria Increase Three-fold an and mitochondrial proteins and lipidchange dramatically in post meristemtial cells in young wheat leaves grown in elevated CO$_2$. Plant Physiol. 1995b;108:469-474.

21. Spurr AR. A low viscosity epoxy resin-embedding medium for electron
microscopy. J. Ultrastruct. Res. 1969;26:31-43.

22. Sharma, Neha Sinha PG, Bhatnagar AK. Effect of elevated CO2 on cell structure and function in seed plants Climate Change and Environmental Sustainability. 2014;2(2):69-104. DOI:10.5958/2320-642X.2014.00001.5

23. Zheng Y, Li F, Hao L, et al. Elevated CO2 concentration induces photosynthetic down-regulation with changes in leaf structure, non-structural carbohydrates and nitrogen content of soybean. BMC Plant Biol. 2019;19:255. Available:https://doi.org/10.1186/s12870-019-1788-9

24. Masle L. The effects of elevated CO2 concentration on cell division rates, growth pattern and blade anatomy in young wheat plants are modulated by factors related to leaf position, vernalisation and genotype. Plant Physiol. 2000;122:1399-1415.

25. Ferris R, Sabatti M, Migletta F, Mills R, Taylor G. Leaf area is stimulated in poplar by free air CO2 enrichment (POPFACE), through increase cell expansion and production. Plant Cell Environ. 2001;24:305-315.

26. Thomas JF, Harvey CN. Leaf anatomy of four species grown under continuous CO2 enrichment. Bot.Gaz. 1983;144:303-309.

27. Vaz M, Cochard H, Gazarini L, Graça J, Chaves MM, Pereira JS. Cork oak (Quercus suber L.) seedlings accclimate to elevated CO2 and water stress: Photosynthesis, growth, wood anatomy and hydraulic conductivity. Trees. 2012;26:1145–1157.

28. Xu CY, Salih A, Ghannoum O, Tissue DT. Leaf structural characteristics are less important than leaf chemical properties in determining the response of leaf mass perarea and photosynthesis of Eucalyptus saligna to industrial-age changes in [CO2] and temperature. J. Exp. Bot. 2012;63:5829–5841.

29. Wei M, Xing XY, Wang XF, Ma H. Effects of CO2 enrichment on the micro structure and ultrastructure of leaves in cucumber. ActaHort.Sin. 2002;29:31–34.

30. Kumar GK, Guha A, Reddy AR. Elevated CO2 atmosphere significantly increased photosynthesis in a fast growing tree species, Gmelina arborea Roxb. Climate Change and Environmental Sustainability. 2013;1:81–94.

31. Zhang F, Wang Y, Huang Z, Zhu X, Zhang F, Chen F, Fang W, Teng N. Effects of CO2 enrichmenton growth and development of Impatiens hawkeri. The Scientific World Journal. 2012;1:1–9.

32. Uprety DC, Dwivedi N, Jain V, Mohan R. Effect of elevated carbon dioxide concentration on the stomatal parameters of rice cultivars. Photosynthetica. 2002;40:315–319.

33. Vu JCV, Baker JT, Pennanen AH, Allen LH Jr, Bowes G, Boote KJ. Elevated CO2 and water deficit effect on photosynthesis, ribulosebisphosphatecarboxylase- oxygenase and carbohydrate metabolism in rice. Physiol. Plant. 1998;103:327-339.

34. Ferris R, Taylor G. Elevated CO2 water relations and biophysics of leaf expansion in four chalk grassland herbs. New Phytol. 1994;127:297-307.

35. Lee H, Evans L, Centritto M, Jarvis P. The effects of elevated CO2 on growth and physiology of birch (Betula pendula Roth). Ecophysiology and genetics of trees and forests in a changing environment. 1. Session. Global change and forest ecosystems. Viterbo (Italy).23–30 May1993. Agricoltura-Ricerca(Italy). 1993;15(145):50.

36. Luo Y, Field CB, Mooney HA. Predicting response of photosynthesis and root fraction to elevated CO2 a: Interactions among carbon, nitrogen and growth. Plant Cell Environ. 1994;17:1195-1204.

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