BRIEF COMMUNICATION

Effect of elevated CO_2 concentration on leaf structure of *Brassica juncea* under water stress

D.C. UPRETY, N. DWIVEDI, R. MOHAN and G. PASWAN

Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi-110012, India

Abstract

Study on the effect of elevated CO_2 concentration on leaf structure of *Brassica juncea* L. cv. Bio-141 (95) under moisture stress revealed, that CO_2 elevated to 600 μmol mol^{-1} increased the length of epidermal cells and the number and length of palisade parenchyma cells, and induced larger chloroplasts and more oval shaped starch granules in comparison with plants grown at ambient CO_2 concentration. This increase in structural sink size helped in checking feedback inhibition by excessive photoassimilate which was subsequently used to compensate the adverse moisture stress effect in *B. juncea* leaves.

Additional key words: chloroplasts, epidermal cells, open top chamber, palisade parenchyma cells, starch granules.

The rising concentration of CO_2 in the atmosphere is one of the important global climate change which affects the productivity of crop plants (Lawlor and Mitchell 1991). Most of the previous investigations done in *Brassica* species were focussed on physiological processes such as photosynthesis, dry matter production and yield (Uprety *et al.* 1995, Uprety and Rabha 1999, Uprety *et al.* 2000, Uprety and Mahalaxmi 2000). Uprety *et al.* (1995) reported the increased drought resistance in *Brassica* species due to elevated CO_2 and attributed it to greater photosynthetic efficiency and improved water status. The implications of elevated CO_2 in crops may also lead to alterations in anatomical structure (Cave *et al.* 1981). Such investigations on anatomical responses are very limited (Radoglou and Jarvis 1992). These studies are required as additional evidences to explain the physiological changes in leaves, particularly in relation to water stress. The objective of the present study was to identify the changes in the epidermal cells, palisade cells, chloroplasts and starch granules of the leaf using transmission electron microscopy (TEM) in *Brassica juncea* grown in open top chambers (OTC) with ambient and elevated CO_2 concentration, under irrigated and moisture stress conditions.

Brassica juncea L cv. Bio-141 (95) was grown in OTC of 2.6 m diameter, lined with transparent PVC sheets. Moisture stress treatment was given by withholding irrigation for 8 d between first flower initiation to 50 % flowering to reach soil moisture 8 to 10 %. Control plants were grown at soil moisture 23 to 25 %. CO_2 enrichment to 600 ± 20 μmol mol^{-1} was done by continously injecting 100 % CO_2 in the input blower where it was mixed with ambient air before entering the chamber. The flow of gas was regulated using solenoid valve, flowmeter and pressure gauge. One thermo-hygrograph was kept in each OTC, to record changes in temperature and relative humidity in the chambers. The cultivation and CO_2 enrichment were done similarly as reported by Uprety and Rabha (1999). Three replicates of each treatment were taken for every observations. This study was done in 1998 and 1999. The results of both the years were similar and the observations of 1999 are presented here.

Slices (2 to 3 mm thickness) were cut from the intact leaf tissue of the fully expanded uppermost leaf at the flowering stage, *i.e.* 45 d after sowing. These slices were fixed in 0.1 M phosphate buffer (pH 7.2) for 1 h at room temperature. Leaf tissues were post-fixed in 1 % (v/v) osmium tetraoxide solution in 0.1 M phosphate buffer for 2 h at room temperature. These tissues were dehydrated through an acetone series and embedded in resin. Ultra-thin sections were cut, using a silver knife and mounted

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Fax: (+91) 011 5766420, e-mail: uprety@vsnl.net

on 200 square mesh copper grids. Double staining of the sections was done by uranyl acetate and lead citrate before examination on an electron micrograph (Robertson *et al.* 1995). Following anatomical parameters of the leaf were studied: length of epidermal and palisade parenchyma cells, chloroplasts and starch granules and number of starch granules per chloroplast, their shape and number of cells per epidermal and mesophyll section area units. Data were analysed statistically following the method of analysis of variance (Snedecor and Cochran 1980).

Significantly higher length of epidermal cells (50 %), palisade cells (78 %), chloroplasts (40.4 %) and starch granules (11.5 %) was observed in plants grown at elevated CO_2 concentration in comparison with plants grown at ambient CO_2 concentration. The number of starch granules per chloroplast increased up to 140 %. The size of starch granules was changed from flat to eliptical and from eliptical to oval (Figs. 1 - 3).

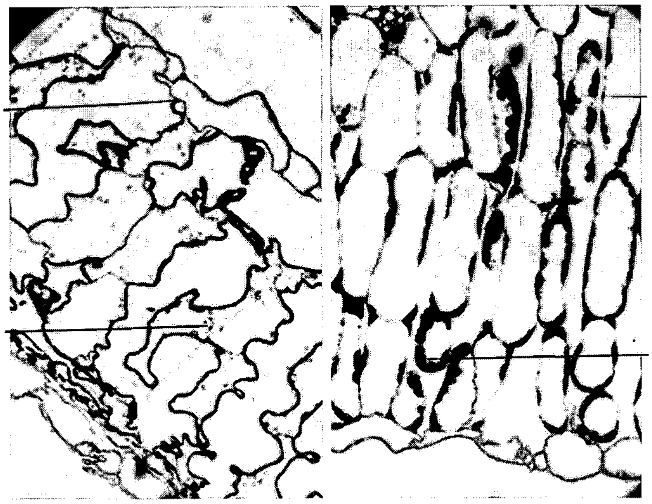

Fig. 1. Effect of elevated CO_2 concentration on the cell anatomy of *Brassica juncea* leaves under water stress.

Table 1. Effect of elevated CO_2 [μmol mol^{-1}] concentration and water stress on length [μm] of epidermal cells, palisade cells, chloroplasts and starch granules, number of epidermal and palisade cells per unit leaf area [mm^{-2}], and number of starch granules per chloroplast in *Brassica juncea* leaves.

CO_2 concentration	Stress	Length epidermal cells	palisade cells	chloroplasts	starch granules	Number of cells epidermal	palisade	Number of starch granules
360	control	52.0	180.0	8.7	1.70	27	16	2 - 3
	stress	38.5	75.0	6.7	1.30	24	10	1 - 2
600	control	70.0	260.0	11.2	1.90	28	22	5 - 6
	stress	66.0	195.0	10.6	1.70	26	19	5
CD at $P = 5$ %	CO_2	4.72	6.25	0.64	0.31	n.s.	1.56	-
	stress	4.72	6.25	0.64	0.31	n.s.	1.56	-
	interaction	n.s.	7.20	0.86	0.39	n.s.	n.s.	-

Moisture stress brought about reduction in the length of epidermal cell (26 %), palisade cell (58 %), chloroplasts (23 %) and starch granules (27 %) under ambient CO_2 concentration. The reduction in number of starch granules per chloroplast was 40 %. However, the reduction was significantly lower under elevated CO_2 concentration and remained only 6 % in epidermal cell, 25 % in palisade cell, 5 % for chloroplast and 10.5 % for starch granule. The reduction in starch granule numbers per chloroplast was 10 % (Table 1). The upper and lower epidermis tended to be thicker in leaves that developed at high CO_2. The mesophyll cell thickness was significantly greater in elevated CO_2 grown leaves as compared to ambient one. The chloroplasts developed under high CO_2 were located more along the cell wall. The starch accumulation was larger under elevated CO_2 than under ambient one. The chloroplasts under high CO_2 had more and larger starch grains. The number of epidermal cells per unit area was not significantly affected by CO_2 concentration. It appears that by increasing the number and size of palisade cells and concomitantly the number of chloroplasts per leaf, more storage sites for starch were made available under stress condition which were otherwise lacking such sites and sink capacity for starch accumulation. The adverse effect on chloroplast structures both due to moisture stress and by starch overloading was ameliorated by elevated CO_2. The production of photoassimilates under elevated CO_2 might increase the sink capacity of leaf blade and consequently reduce the moisture stress effect. The feedback inhibition did not occur because of the larger palisade and epidermal cells which compensate the adverse water stress effect on these components. However, there was no significant increase in the number of epidermal cells per unit leaf area in high CO_2 treatment.

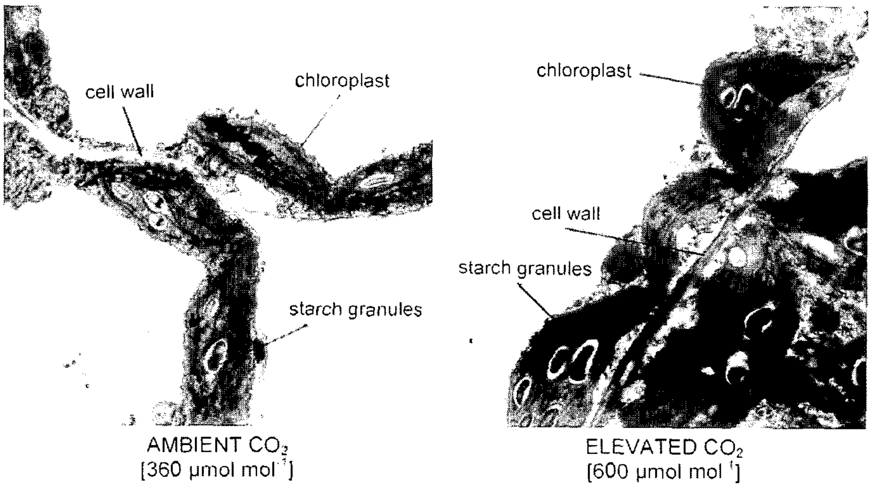

Fig. 2. Effect of elevated CO_2 concentration on ultrastructure of *Brassica juncea* leaves under water stress.

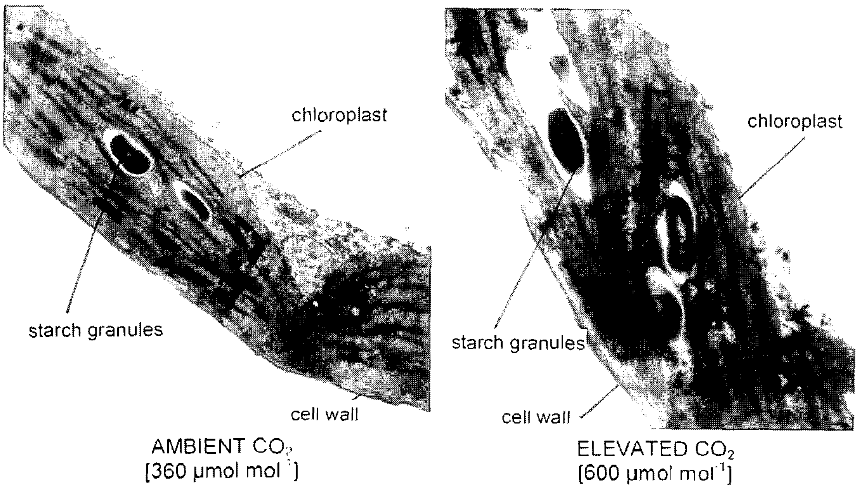

Fig. 3. Effect of elevated CO_2 concentration on ultrastructure of *Brassica juncea* leaves under water stress (details).

The leaf thickness was affected by increase in the length of epidermal cells and increase in number and elongation of mesophyll cells. It seems that cell enlargement was more sensitive to CO_2 than cell division and this may be related to changes in leaf water relations (Uprety *et al.* 1995). It appears that leaf anatomy reflected an optimization strategy due to high CO_2 that allows the loading of chloroplast with excessive oval starch grains to meet the adverse effect of drought on these components in *Brassica juncea* leaves.

References

Cave, G., Tolly, L.C., Strain, B.R.: Effect of CO_2 enrichment on chlorophyll content, starch content and starch grain structure in *Trifolium subterraneum* leaves. - Physiol. Plant. **51**: 171-174, 1981.

Lawlor, D.W., Mitchell, A.C.: The effects of increasing CO_2 on crop photosynthesis and productivity: a review of field

studies. - Plant Cell Environ. **14**: 807-818, 1991.
Radoglou, K.M., Jarvis, P.G.: The effects of CO_2 enrichment and nutrient supply on growth, morphology and anatomy of *Phaseolus vulgaris* L. seedlings. - Ann. Bot. **70**: 245-256, 1992.
Robertson, E.J., Williams, M., Harwood, J.L., Gordon, L., Leaver, C.J., Leech, R.M.: Mitochondria increase three fold in post meristematic cells in young wheat leaves *Triticum aestivum* grown in elevated CO_2. - Plant Physiol. **108**: 469-474, 1995.
Snedecor, G.W., Cochran, W.G.: Statistical Methods. 7th Ed. - Iowa State Press, Ames 1980.
Uprety, D.C., Garg, S.C., Tiwari, M.K., Mitra, A.P.: Crop responses to elevated CO_2: technology and research (Indian studies). - Global Environ. Res. J. **3**: 155-167, 2000.
Uprety, D.C., Mahalaxmi, V.: Effect of elevated CO_2 and nitrogen nutrition on photosynthesis, growth and carbon-nitrogen balance in *Brassica juncea*. - J. Agron. Crop Sci. **184**: 271-276, 2000.
Uprety, D.C., Mishra, R.S., Abrol, Y.P.: Effect of elevated CO_2 on the photosynthesis, growth and water relation of *Brassica* species under moisture stress. - J. Agron. Crop Sci. **175**: 231-237, 1995.
Uprety, D.C., Rabha, B.K.: Effect of elevated CO_2 and moisture stress on the carbon and nitrogen contents in *Brassica juncea*. - Biol. Plant. **42**: 133-136, 1999.