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EFFECT OF ELEVATED CO_2 ON WATER RELATION COMPONENTS OF *BRASSICA* SPECIES UNDER MOISTURE STRESS CONDITION

RANJAN DAS[1] AND D.C. UPRETY*

Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi–12

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SUMMARY

An attempt was made to study the interactive effect of elevated CO_2 and moisture stress on water relations of *Brassica* species grown in FACE facility. It was observed that plants respond to elevated CO_2 significantly under moisture stress condition mitigating the adverse effects on leaf relative water content and intercellular water movement. The water status of plants improved under elevated CO_2 concentration possibly by increasing water and osmotic potential due to increase in nonstructural carbohydrates, free amino acids and free fatty acids.

***Key words*: *Brassica*, elevated CO_2, FACE technology, inter cellular water movement, water status**

INTRODUCTION

The CO_2 concentration in the atmosphere is rising and is likely to be doubled by the end of the next century. The rise in atmospheric concentration of CO_2 has major effect on agriculture through regional changes in air temperature, humidity, and length of growing season, precipitation pattern and evapotranspiration, all of which affect plant water relations. An attempt has been made in the present study to analyze the effect of elevated CO_2 on the plant water status in *Brassica*, an important oil seed of north-western India. This crop experiences intermittent drought during its growth period from vegetative to the siliqua formation stage, and there was variability in the responses of *Brassica* cultivars to the moisture stress (Chopra 1991 and Uprety *et al*. 1995). It was therefore, considered important to study the interactive effect of the elevated CO_2 and moisture stress, on the water relation parameters of *Brassica* species in the present investigation.

MATERIALS AND METHODS

Brassica cultivars, *viz*. *Brassica juncea* cv. RH-30 and *Brassica campestris* cv. Pusa Gold were grown in the field inside the Mid Free Air CO_2 Enrichment (FACE) facility in eight metres diameter circle during the winter crop season (Rabi season) of 2001 and 2002. The Free Air CO_2 Enrichment (FACE) technology was based on the principle of injecting additional CO_2 gas in open field, 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.

An elevated CO_2 concentration of 550µmol mol^{-1} was maintained with the help of computer based proportional integral differential (PID) valves, which controlled the quantity of CO_2 to be released into the arms throughout the growth period. Ambient condition was maintained without any exogenous supply of CO_2 to the

* Corresponding author
[1]Present address: Department of Crop Physiology, Assam Agri. Univ., Jorhat 785013, Assam

normal air under field condition. Recommended package of practices were followed (Uprety *et al* 2002). Moisture stress treatment was given by restricting irrigation. The average moisture content of soil was 23-25 per cent under irrigated condition and it was maintained between 8-10 per cent under moisture stress condition during crop growth period. Observations were recorded at vegetative (25 DAS), flower bud initiation (45 DAS), 50% flowering (60 DAS) and post flowering stages. Soil moisture content was measured using gravimetric method (Dastane 1972).

Sample extraction for estimation of sugar was done according to method reported by McCready *et al.* (1950). The reducing sugar was determined by Nelson's arsenomolybdate method (Nelson 1944). The reducing sugar was subtracted from the total sugar to obtain the non-reducing sugar content (Allen *et al*. 1988). Starch content was determined by Anthrone method (Mcready *et al*. 1950.) Free amino acid (FAA) content of leaves was estimated spectrophotometrically following the method of Lee and Takahashi (1966). Free fatty acid (FFA) of the leaf sample was initially extracted in 4N HCl and then with chloroform. Total free fatty acid was separated and estimated following the method of Thimmaiah (1999). The amount of free fatty acid was calculated by using standard curve prepared from palmitic acid.

Uppermost fully expanded fresh leaves of main stem were collected at 10 a.m. to study the water relation parameters at different stages of growth. Four replications were taken from each treatment. Twenty discs were punched from the leaves of each treatment and weighed. Leaf discs were submerged in water in a petridish for four hours. They were blotted and their saturated weight was measured. Discs were dried at 80 ^{0}C till constant weight. Relative water content (RWC) was calculated using under mentioned equation and expressed as percentage. (Weatherly and Barrs 1962)

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

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

Sap from fresh leaves was extracted by squeezing leaf samples. The extracted sap was used as osmol. The concentration of osmol (M) was determined by vapor pressure osmometer (Model 5500, Wescor, Inc, USA). The osmotic potential of samples was determined following the formula suggested by Wyne Jones and Graham (1983) and was expressed in MPa.

$$(\Psi s) = MxT \times 0.00832$$

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

Intercellular water movement

Fully expanded fresh leaf (6th node from the top) of main stem was collected in the forenoon at full sunlight (PPFD~1500 µmol $m^{-2}s^{-1}$) to study the intercellular water movement by Nuclear Magnetic Resonance (NMR) technology. The samples were rolled into a cylinder (2 cm height) to fit tightly into a 1.8 cm diameter NMR sample tube for measurement of T_1. Proton spin-lattice relaxation (T_1) values were measured by 90^0-τ- 90^0pulse sequence using Bruker minispec pc-20 spectrometer (20 MHz) at 25^{0}C temperature. The non-exponential magnetization was resolved by semi-logarithmic graphic method (Tewari *et al.* 1986). The reactive magnitude of magnetization and corresponding relaxation time of each fraction were calculated using equation suggested by Colire *et al*. (1988).

$$M(t) = Mo\,(1-\Sigma_1^2 = {}_1P_i\ e^{-t/T^{1i}})$$

Where, M (t) = longitudinal magnetization at any time t, Mo = equilibrium magnetization, Pi= is the fraction of total spin population present at any time in the ith phase.

The relaxation rate ($R_1=1/T_1$) was calculated from the measured mean value of T_1 under irrigated and moisture stress condition.

RESULTS

CO_2 enrichment significantly increased the non-structural carbohydrate (NSC) content in leaves of *Brassica* cultivars ranging from 21% (vegetative) to 26% (flowering) (Table 1). NSC was more in RH-30. Moisture stress brought about significant reduction in the non-structural carbohydrates ranging between 26% (vegetative) to 45% (flowering). This reduction under ambient CO_2 varied between 31% (vegetative) to 39% (flowering) and under elevated CO_2 20% (vegetative) to 31% (flowering) in *B. campestris* Pusa Gold. In *B. juncea* RH-30 the reduction was between 29% (vegetative) to 32% (flowering) under ambient and 11% (vegetative) to 25% (flowering) under elevated CO_2 concentration.

CO_2 enrichment did not affect the total free amino acid content in leaves of *Brassica* (Table 2). Free amino acids were higher in RH-30. Moisture stress treatment significantly increased the free amino acids from 1.6-fold (vegetative) to 2.3-fold (post flowering) throughout the growth period. This increase under ambient and elevated CO_2 conditions varied between 1.1-fold (flower bud initiation) to 1.6-fold (post flowering) and 3.0-fold (flower bud initiation) to 3.9-fold (vegetative) respectively in *B.campestris* Pusa Gold and from 1.2-fold (flower bud initiation) to 1.6-fold (post flowering) and 2.9-fold (flower bud initiation) to 6.0-fold (post flowering) respectively in *B.juncea* RH-30.

CO_2 enrichment significantly increased the free fatty acids content in leaves of *Brassica* ranging between 24 to 26 per cent (Table 3). The free fatty acids content was more in leaves of RH-30 compared to Pusa Gold. Moisture stress significantly increased the free fatty acid content ranging between 39% (vegetative) to 54% (flowering). The stress induced increase in free fatty acids under ambient and elevated CO_2 varied between 14% (vegetative) to 29% (flowering) and 46% (vegetative) to 75% (flowering) respectively in Pusa Gold, whereas, this increase ranged between 18% (post flowering) to 30% (flowering) and 67% (vegetative) to 79% (flowering) respectively in RH-30.

Table 1. Interactive effect of elevated CO_2 and moisture stress on non-structural carbohydrates (mg g^{-1}dw) at different stages of growth in *Brassica*

Treatments	Vegetative		Flower bud initiation		Flowering		Post flowering	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE IRR	181.76	181.08	228.82	279.37	270.72	332.96	207.05	247.06
FACE MS	144.70	161.29	170.32	217.51	184.87	246.91	155.62	194.99
Ambient IRR	155.25	173.04	176.17	220.40	194.96	249.34	164.24	196.99
Ambient MS	107.33	122.47	116.54	149.52	118.73	169.30	108.69	139.99
CV.	13.33		23.67		44.78		21.45	
CO_2	16.34		19.44		22.67		16.77	
CV. x CO_2	23.31		25.21		32.06		21.47	
MS	14.63		13.56		27.54		15.44	
CV. x MS	21.34		19.81		30.94		19.89	
CO_2 x MS	23.33		22.24		38.94		24.77	
CV. x CO_2 x MS	30.56		27.12		55.07		30.22	

IRR= Irrigation, MS= Moisture stress, CV= Cultivars

Table 2. Interactive effect of elevated CO_2 and moisture stress on total free amino acid content (mg g^{-1}dw) at different stages of growth in *Brassica*

Treatments	Vegetative		Flower bud initiation		Flowering		Post flowering	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	1.45	1.67	3.12	4.22	4.0	5.07	1.65	2.61
FACE moisture stress	5.64	7.11	9.45	12.21	14.21	16.99	5.91	15.22
Ambient irrigated	2.91	3.67	6.71	7.36	8.24	9.62	3.14	7.1 0
Ambient moisture stress	3.95	4.95	7.42	8.72	9.79	11.94	4.72	11.20
CV.	0.72		1.31		1.01		3.33	
CO_2	NS		NS		NS		NS	
CV. x CO_2	NS		NS		NS		NS	
MS	0.24		0.12		0.34		0.65	
CV. x MS	0.45		0.33		0.67		0.87	
CO_2 x MS	0.67		0.43		0.78		1.02	
CV. x CO_2 x MS	0.97		0.62		1.21		1.32	

Table 3. Interactive effect of elevated CO_2 and moisture stress on free fatty acid content (mg g^{-1}dw) at different stages of growth in *Brassica*

Treatments	Vegetative		Flower bud initiation		Flowering		Post flowering	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE IRR	3.28	4.42	3.87	6.20	5.26	7.21	3.08	6.99
FACE MS	4.79	7.41	6.17	10.65	9.21	12.95	4.52	11.87
Ambient IRR	3.12	4.19	3.75	5.95	5.14	6.87	2.99	6.79
Ambient MS	3.62	5.07	4.59	7.07	6.64	8.90	3.51	8.04
CV.	1.01		2.09		1.67		3.71	
CO_2	0.12		0.02		0.99		0.12	
CV. x CO_2	0.98		0.06		1.40		0.50	
MS	0.34		0.04		0.52		0.29	
CV. x MS	0.67		0.08		0.74		0.45	
CO_2 x MS	0.89		1.02		0.84		0.76	
CV. x CO_2 x MS	1.07		1.34		1.12		0.97	

The elevated level of CO_2 brought about significant increase in the proton spin-lattice relaxation time in leaves of *Brassica* cultivars (Table 4). This increase ranged between 26% (vegetative) to 31% (flower bud appearance). Proton spin-lattice relaxation time was significantly more in RH-30 compared to Pusa Gold. Moisture stress brought about significant reduction in proton spin-lattice relaxation time from 28% (vegetative) to 43% (flowering). This reduction under ambient and elevated CO_2 condition varied between 24% (vegetative) to 34% (flowering) and 16% (vegetative) to 20% (flowering) respectively in Pusa Gold and between 27% (vegetative) to 31% (post flowering) and 13% (post flowering) to 18% (flower bud initiation) respectively in RH-30.

The elevated CO_2 caused significant increase in water potential of leaves ranging between 31% (vegetative) to 37% (flowering) (Fig. 1). Water potential was more in the leaves of RH-30. Moisture stress significantly decreased the water potential from 33% (Post flowering) to 61% (flowering). The stress-induced reduction under ambient and elevated CO_2 conditions varied between 37% (vegetative) to 48% (post flowering) and 25% (vegetative) to 27% (flower bud initiation) respectively in Pusa Gold, whereas, between 33% (vegetative) to 39% (post flowering) and 16% (flowering) to 18% (post flowering) respectively in RH-30.

CO_2 enrichment significantly enhanced the osmotic potential in leaves of *Brassica* cultivars between 22% (flower bud initiation) to 54% (post flowering) (Fig. 2). Osmotic potential was more in RH-30. Moisture stress treatment significantly decreased the osmotic potential of leaves. The reduction varied from 37% (post flowering) to 70% (flowering). The stress-induced reduction under ambient and elevated CO_2 conditions varied between 48% (vegetative) to 62% (flowering) and 25% (vegetative) to 33% (flowering) respectively in Pusa Gold, whereas, in RH-30 it ranged between 35% (flower bud initiation) to 45% (post flowering) and 16% (flowering) to 20% (post flowering) respectively.

The elevated CO_2 brought about significant increase in RWC in the leaves of *Brassica* species ranging from 12% (vegetative and post flowering) to 14% (flowering)

Table 4. Interactive effect of elevated CO_2 and moisture stress on proton pulse relaxation time ($T_{1,}$ ms) in leaves at different stages of growth in *Brassica*

Treatments	Vegetative		Flower bud initiation		Flowering		Post flowering	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	334	390	400	460	410	580	330	520
FACE moisture stress	279	326	320	375	327	480	277	450
Ambient irrigated	283	332	335	380	340	485	280	451
Ambient moisture stress	204	240	220	261	222	342	202	339
CV.	32.43		16.42		59.34		59.23	
CO_2	23.44		8.31		27.44		30.44	
CV. x CO_2	47.54		11.76		49.33		49.21	
MS	23.76		8.09		50.02		26.67	
CV. x MS	44.36		11.44		66.32		42.89	
CO_2 x MS	56.32		13.67		78.11		54.56	
CV. x CO_2 x MS	67.44		17.89		87.44		68.49	

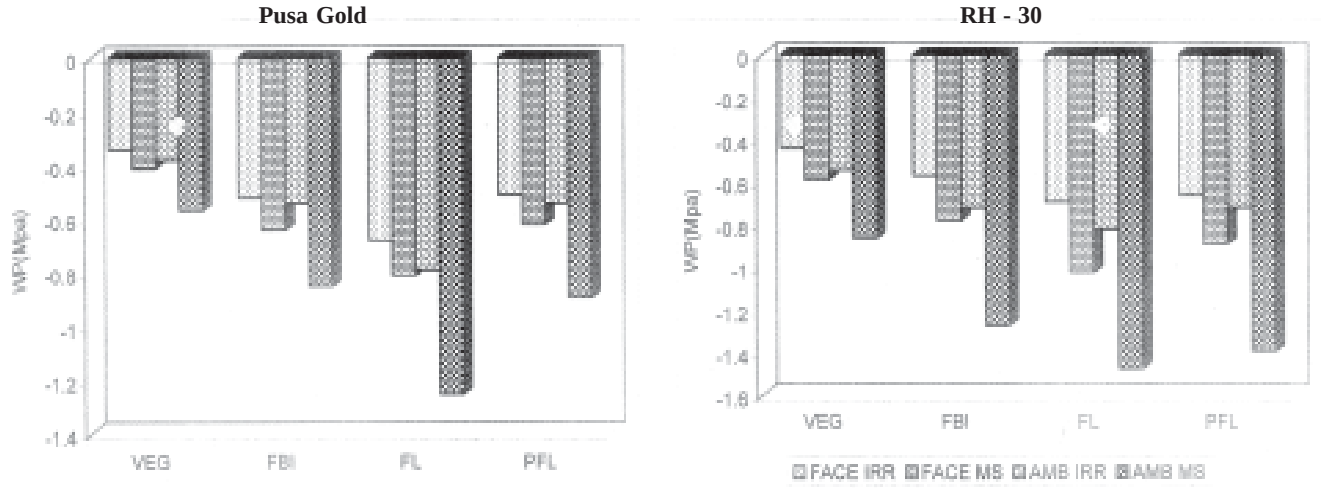

Fig. 1. Interactive effect of elevated CO_2 and moisture stress on water potential (MPa) at different stages of growth in *Brassica*

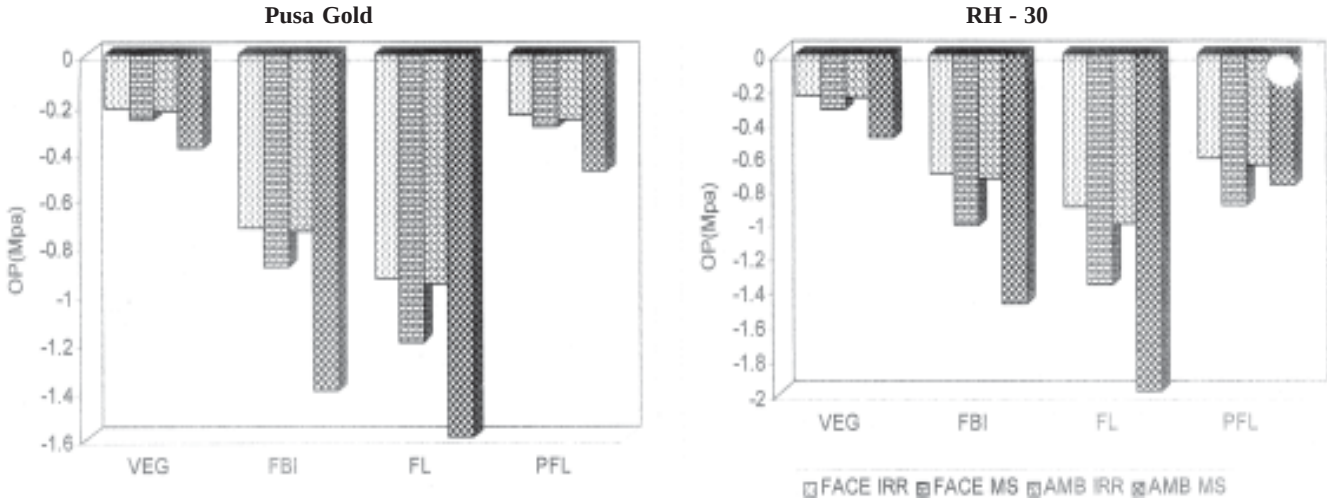

Fig. 2. Interactive effect of elevated CO_2 and moisture stress on osmotic potential (MPa) at different stages of growth in *Brassica*

(Table 5). RWC was more in the leaves of RH-30. Moisture stress significantly decreased the RWC between 16% (flowering) to 11% (post flowering). The stress induced decrease under ambient condition in Pusa Gold was 21% (flowering) to 12% (post flowering), whereas, in RH-30 it was between 24% (flowering) to 16% (post flowering). The stress induced reduction ranged between 6 to 9 % under elevated CO_2 condition in both the cultivars.

DISCUSSION

Elevated CO_2 brought about the accumulation of soluble sugars as well as non-structural carbohydrates, free amino acids and free fatty acids in the cell-sap which helped in the maintenance of osmo-regulation in *Brassica* leaves under moisture stress condition. The higher level of carbohydrates play a protective role against the photo-assimilate shortage during stress. These may contribute to the ameliorating effect of elevated CO_2 to drought stress responses in the present investigation. Read *et al*. (1997) demonstrated that the CO_2 induced accumulation of fructans in canola did not interfere with the photosynthetic apparatus and may possibly used for osmoregulation. According to Ellsworth (1999) elevated CO_2 may directly alter leaf dehydration tolerance in cases where CO_2 induced excess carbohydrates serve as osmotica. Elevated CO_2 could increase drought tolerance

Table 5. Interactive effect of elevated CO_2 and moisture stress on relative leaf water content (%) at different stages of growth in *Brassica*

Treatments	Vegetative		Flower bud initiation		Flowering		Post flowering	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	75.47	83.60	80.90	89.92	77.00	88.06	67.00	79.76
FACE moisture stress	71.57	76.90	74.00	84.00	72.12	80.60	61.90	72.86
Ambient irrigated	74.00	79.40	77.80	87.10	74.40	84.60	60.64	77.27
Ambient moisture stress	69.20	71.02	71.39	76.03	68.08	70.92	56.02	70.01
CV.	6.85		4.52		5.02		5.49	
CO_2	6.49		6.65		5.50		4.79	
CV. x CO_2	9.18		9.41		7.T8		6.37	
MS	3.17		3.34		3.71		3.97	
CV. x MS	4.31		4.82		5.25		5.20	
CO_2 x MS	6.89		7.14		7.42		7.94	
CV. x CO_2 x MS	8.61		9.63		10.49		10.51	

of plants if increased rates of net carbon assimilation leads to the availability of substrate for osmotic adjustment. Elevated CO_2 has significant effect on drought tolerance by inducing carbohydrate accumulation for growth and by enhancing osmotic adjustment during drought (Wullschleger *et al.* 2002). Carbon dioxide enrichment did not affect the free amino acid content in *Brassica* species. Moisture stress significantly increased the free amino acid content. Significantly higher amount of free amino acids was observed due to the interactive effect of elevated CO_2 and moisture stress. These amino acids may possibly play an important role in the osmo-regulation under stress condition. Elevated CO_2 also brought about significant increase in free fatty acid content in the leaves of *Brassica* cultivars throughout the growth period. Moisture stress significantly increased the free fatty acid content. The interactive effect of elevated CO_2 and moisture stress also significantly increased the free fatty acid content. These fatty acids were released by the action of lipolytic enzymes in leaves of *Brassica oleracea* plant when subjected to water stress (Cheour *et al*. 1992) and involved in metabolite adjustment during stress (Aziz and Larher 1998).

The adverse effect of moisture stress on water status of *Brassica* leaves might be counteracted due to increase in the random distribution of water molecules and more free available water for participation in metabolic activities under elevated CO_2 condition, which was confirmed by Nuclear Magnetic Resonance (NMR) data in the present study. NMR offers a non-destructive and non-invasive method for characterizing tissue water status (Mathur, DeVre 1979). Colire *et al*., (1988) have shown that spin-lattice relaxation time (T_1) of leaf water was closely related to classical water status parameters such as RWC, water potential and solute potential in barley leaves. According to them, it determines the internal water availability, its physiological compartmentation, its states, and the variations under stress. The higher T_1 implies a more random distribution of water molecules and free availability of water for participation in metabolic activities (Lewin 1974). In the present study, T_1 increased significantly in leaves of *Brassica* cultivars throughout the growth period. Higher-level proton spin-lattice relaxation time (T_1) was observed in RH-30. Moisture stress decreased the proton spin-lattice relaxation time and this reduction was lesser under

elevated CO_2. Uprety *et al*. (1995) also demonstrated that elevated CO_2 partially ameliorated the negative effects of moisture stress in *Brassica* spp. by increasing the water potential, apoplastic water and regulating turgor through positive changes in tissue elasticity. In the present investigation, the water status of *Brassica* leaves was affected more through changes in the dynamic phase of water (water potential and mobility of water molecules).

Centritto *et al*. (1999) attributed the delay in the onset of drought under elevated CO_2 to the decline in stomatal conductance, reduction in transpiration, and also to increase in plant water potential. These componental changes were responsible to maintain the higher plant water potential under moisture stress condition enabling plants to remain turgid and functional for longer time. Elevated CO_2 significantly increased the water status of the leaves in *Brassica* by affecting water relation parameters. The water potential, osmotic potential, and relative water content were increased significantly in leaves of *Brassica* cultivars throughout the growth period. Moisture stress adversely affected these water relation parameters. The stress-induced effect on these parameters significantly ameliorated in higher level of CO_2. Besides adjusting the regulation of water in plants through changes in various water relation components, CO_2 enrichment also helped in osmotic adjustment through the accumulation of fructans, free amino acids and free fatty acids in *Brassica* species under moisture stress condition. These adjustments protected *Brassica* plants from the adverse stress effects.

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