Research Article

Study on the response of diploid, tetraploid and hexaploid species of wheat to the elevated CO_2

D.C. Uprety, N. Dwivedi, A. Raj, S. Jaiswal, G. Paswan, V. Jain and H.K. Maini

Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi - 110 012, India

ABSTRACT

Study was done to compare the response of *Triticum aestivum* (hexaploid), *Triticum durum* (tetraploid) and *Triticum monococcum* (diploid) wheat species to the elevated CO_2 using Free Air CO_2 Enrichment (FACE) facility. It was demonstrated that the modern cultivar of wheat *Triticum aestivum* (hexaploid) was largely sink limited. It appeared to have less photosynthesis per unit leaf area than *Triticum monococcum* (diploid wheat). While leaf size, grain weight and amylase activity increased with the ploidy level from diploid to hexaploid wheat forms, the photosynthetic rate was reduced significantly. These wheat species responded differentially to the elevated CO_2. The larger leaf area and greater seed weight and presence of 38 KDa protein band caused by elevated CO_2 had additive effect in improving the productivity of hexaploid wheat by changing the source sink ratio. Whereas, such a source sink balance was not induced by elevated CO_2 in diploid wheat. The increasing CO_2 may present opportunities to breeders and possibly allow them to select for cultivars responsive to the elevated CO_2 with better sink potential. **[Physiol. Mol. Biol. Plants 2009; 15(2) : 161-168]** *E-mail : upretydc@gmail.com*

Key words : *Elevated CO_2, FACE technology, Photosynthesis, Seed weight, Source sink ratio, Triticum*

INTRODUCTION

Human activities have caused the concentration of atmospheric CO_2 to increase continuously from 280-µmol mol^{-1} of pre industrial period to the current level of 376-380 µmol mol^{-1} (Uprety and Reddy 2008). Future projection of atmospheric CO_2 concentration expected to be near double of the present value in the middle of 21st century (IPCC, 2007). The potential consequence of elevated CO_2 is its effect on the process of photosynthesis, which effectively contributes to the productivity of crop plants (Uprety *et al.*, 1995). The increasing CO_2 also causes rise in temperature by absorbing long wave heat radiations. Earlier studies on the effect of elevated CO_2 in crop plants showed significant diversity in the response of maize, *Brassica* and rice cultivars (Uprety *et al*. 1995, 1996 and 2007). This variability in crop responses to the elevated CO_2 made the agricultural productivity and food security vulnerable to the climate change.

Wheat is physiologically and genetically capable of much higher productivity and photosynthetic efficiency than has been recorded in a field environment, indicating their vulnerability to the changes in climatic attributes. The rationale of the present study is to understand the physiological modifications due to increasing CO_2 concentration and higher temperature during the evolution of hexaploid *Triticum aestivum* wheat from their diploid and tetraploid progenitors. Missing information on the responses of diploid and tetraploid species in comparison to hexaploid wheats to the elevated CO_2 will be helpful to explain the adaptations evolved in the hexaploid wheats. The diploid and tetraploid wheats did not experience such a drastic change in the concentration of atmospheric CO_2 during their evolution and their response will explain missing link. Changes occurred due to the addition of genomes during the evolution of hexaploid wheat and increase in the atmospheric CO_2 was analyzed in the current study using FACE technology for CO_2 enrichment.

MATERIALS AND METHODS

Three wheat species namely *Triticum monococcum* (diploid), *Triticum durum* (tetraploid) and *Triticum*

Correspondence and reprint requests : Dr. D.C. Uprety
Ph.: 011-25846107, 9871986313

aestivum (hexaploid , Var. PBW-373) were grown in FACE rings with CO_2 concentration of 550 µmol mol^{-1} and control fields with 376 to 386 µmol mol^{-1} (100 meters away from the FACE rings).These wheat species were procured from National Bureau of Plant Genetic Resources, New Delhi. Seeds were sown on November, 23rd, 2005. The nitrogen, phosphorus and potassium fertilizers were applied as per recommended cultivation practice, i.e. 120: 60: 60 Kg/ha in term of urea, super phosphate and potash respectively in two split applications i.e. at the time of field preparation for sowing and at the crown root stage. The natural day length was extended to 16 hours by fluorescence bulbs (Sodium vapor Philips HLRG 400 W) for diploid wheat *T. monococcum* as per their greater light requirement (Uprety *et al* 1987). Care was taken to check the incursion of additional light to other species. Plants were grown under irrigated condition and irrigation was given after sowing at vegetative, crown root stage, maximum tillering, anthesis, seed filling, and post flowering stages. The monthly average of minimum, maximum temperature and relative humidity are given in the (Table 1).

The Free air CO_2 enrichment technology, first of its kind was established in India at Indian Agricultural Research Institute, New Delhi. Structured in 8-meter diameter octagon shape, FACE ring was modified according to the prevailing climatic conditions of South Asia. The plenum was made up of flexible irrigation pipe having 20 cm diameter. All eight nodes of the octagon have independent control of CO_2 with the help of computer controlled PID valves. CO_2 was injected from 25-gas cylinder storage with manifold, valves and flow meters having a CO_2 regulating system to the input blower for mixing. CO_2 was dispersed in the FACE ring through large number of holes in the pipe. The fumigation of the gas from the plenum was made at the center of the field 10x15 cm above the crop canopy level to reduce CO_2 gradient with depth and to make the CO_2 concentration uniform. The plenum height was adjusted time to time to the height of canopy with the help of adjustable stands. A PC based system controller was used to control the PID valves for maintaining the required CO_2 concentration in these FACE rings. The system controller controls as well as analyses and displays the data on graphic terminals (Uprety *et al.,* 2000 and 2007). (Fig. 1).

The effect of elevated CO_2 on wheat cultivars was similar at different stages of growth. However, the intensity of response was different at different stages. These wheat species showed maximum response at flowering stage. Thus the response of wheat species to the elevated CO_2 at flowering stage is described here for the sake of brevity and to avoid repetition in the description. These wheat species flowered at similar time. Plants were sampled and dried in an oven at 80 °C till constant mass. The dry weight of whole plant biomass was recorded. Leaf area was measured using Licor leaf area meter (Licor 3000). Net photosynthetic rate (P_N), stomatal conductance, chlorophyll content, nitrogen and sugar contents in the youngest fully developed top most leaf of main shoot was measured. Photosynthesis and stomatal conductance were determined by photosynthetic system Licor-6200 (Lincoln, NE, USA) between 10.00 to 11.30 AM at full sunlight.

Total chlorophyll content was estimated using dimethyl sulphoxide (DMSO) by Hiscox and Isaraelstam's method (1976). Nitrogen content was estimated in the dried samples of leaves by modified Kjeldahl method of Bremner, and Mulvaney(1965). Sugars were extracted by the method of McCready *et al.* (1950). The reducing and total sugars were determined colorimetrically by arsenomolybdate method (Nelson 1944). Protein content and protein profile was studied in upper most fully expanded leaves at flowering and in grains from the middle of spikelet of wheat species. These plant samples were ground and extracted with 20 ml of tris- HCl buffer (50 mM, pH 8.8) for one hr at 4 °C with vortexing at 15 min intervals and centrifuged (20,000 g, 20 min). The supernatant containing the soluble proteins was carefully removed and stored at -20 °C. Protein, quantitization

Table 1. Mean monthly minimum, maximum temperature (°C) and relative humidity (%) during the wheat growing period

	NOV 2005	DEC 2005	JAN 2006	FEB 2006	MAR 2006	APRIL 2006
Min temperature °C	10.6	9.3	5.7	9.7	13.8	20.2
Max. Temperature °C	27.4	19.9	16.3	21.7	28.1	37.2
Relative humidity %	56.0	76.0	82.0	75.0	58.0	42.0

Fig. 1. Wheat species grown in Free Air CO_2 enrichment facility

was carried out by the CBG -250 dye binding method of Bradford (1976) using BSA standard. SDS-PAGE analysis was carried out according to Laemmli (1970) as modified by Singh and Shephered (1985). The SDS-gel contained a 4 % poly acrylamide staking gel and a resolving gel of a 10 % polyacrylamide using the buffer system of Laemmli (1970). Proteins were fixed and stained with commassie–brilliant blue (CBB) dye binding method after completion of electrophoresis. Amylase activity in harvested seeds was determined by colorimetric method of Bernfield (1955) using 3, 5 dinitro salicylic acid and phosphate buffer. It was expressed in µg maltose released per µg protein.

Total harvested biomass was sampled and their dry weight was measured. Seed yield and grain weight were recorded from the final harvested material. Harvest index was calculated dividing grain weight by total biomass dry weight. The harvested seeds of diploid (*Triticum monococcum*), tetraploid (*Triticum durum*) and hexaploid (*Triticum. aestivum*) wheats were examined for the grain size using camera attached to a 3 Dimensional Olympus microscope Model.SZ2-1LST) using a computer-assisted image analysis system as reported by Purnobasuki and Suzuki (2005). All the observations were taken in triplicate. Statistical analysis of the data was done following the method of analysis of variance (Snedecor and Cochran, 1972).

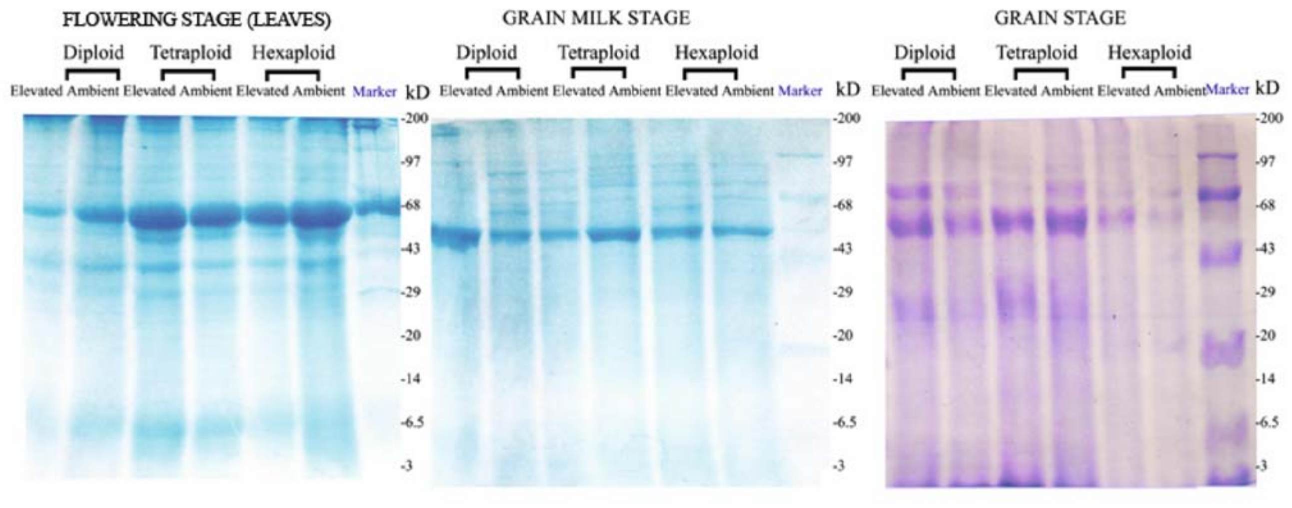


Fig. 2. Effect of elevated CO_2 on the protein profile in the leaves and grains of wheat species.

Table 2. Effect of elevated CO_2 on the morpho-physiological characters of wheat species.

	CO_2 Concentration	Diploid	Tetraploid	Hexaploid	CD at 5% p	
Photosynthesis (µmol m^{-2} sec^{-1})	Elevated	27.2	25.3	23.3	S	2.32
	Ambient	25.2	21.2	19.6	T	1.89
					SxT	NS
Stomatal conductance (mol m^{-2} sec^{-1})	Elevated	0.26	0.22	0.19	S	0.050
	Ambient	0.39	0.35	0.21	T	0.040
					SxT	0.070
Total chlorophyll (mg/g f. wt)	Elevated	0.55	0.75	0.86	S	0.082
	Ambient	0.66	0.96	1.09	T	0.101
					SxT	NS
Leaf Area (cm^{-2} m^{-2})	Elevated	8452	9871	12202	S	342.7
	Ambient	7824	8331	10013	T	279.8
					SxT	484.6
Total Sugar (mg/g)	Elevated	0.925	0.768	0.680	S	0.808
	Ambient	0.649	0.569	0.505	T	0.066
					SxT	NS
Reducing Sugar (mg/g)	Elevated	0.731	0.568	0.497	S	0.065
	Ambient	0.428	0.363	0.300	T	0.053
					SxT	NS
Nitrogen (g/100g)	Elevated	0.807	0.805	0.570	S	0.066
	Ambient	0.965	0.905	0.620	T	0.054
					SxT	NS
Dry weight (g/m^2)	Elevated	1053.4	1452.6	1845.0	S	93.7
	Ambient	867.9	1153.8	1262.0	T	76.5
					SxT	132.5
Grain yield (g/m^2)	Elevated	90.6	446.2	626.0	S	27.3
	Ambient	80.0	385.0	512.3	T	22.3
					SxT	38.6
1000 grain wt. (g)	Elevated	17.8	22.4	30.5	S	2.8
	Ambient	16.9	24.9	28.2	T	NS
					SxT	NS
Harvest Index (%)	Elevated	7.8	30.3	35.5	S	2.5
	Ambient	7.4	27.4	30.9	T	2.08
					SxT	NS
Dry weight at Harvest (g/m^2)	Elevated	1161.53	1472.60	1763.38	S	72.5
	Ambient	1081.08	1405.10	1657.92	T	42.8
					SxT	NS

Elevated = (550 µmol $mol^{-1,}$ CO_2 Concentration), Ambient = (376-386 µmol mol^{-1} CO_2 Concentration) S = species, T = treatment, S×T = species × treatment.

RESULTS

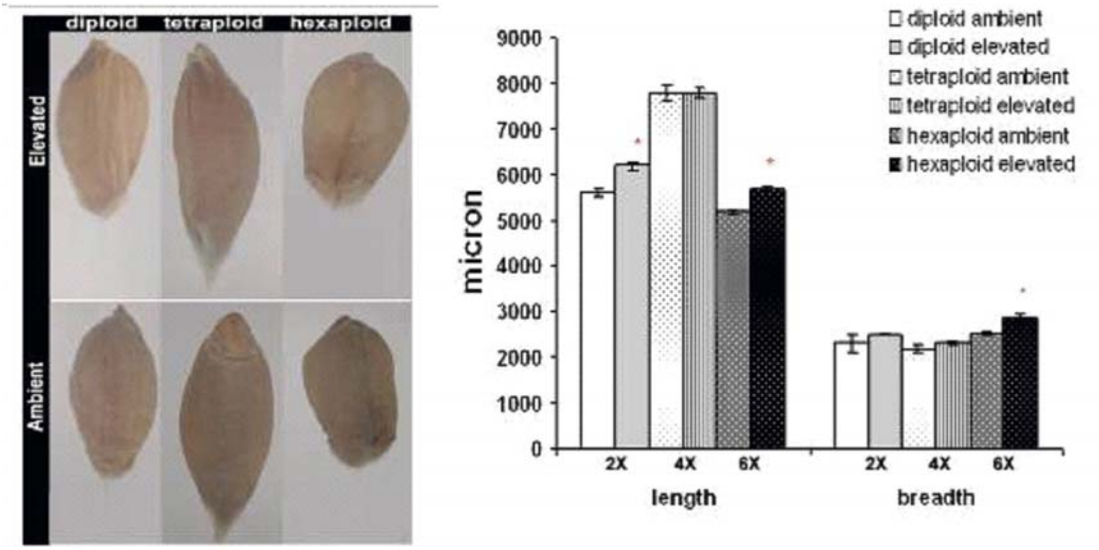
The rate of photosynthesis was significantly higher in diploid (*Triticum monococccum*) compared to tetraploid (*Triticum durum*) and hexaploid (*Triticum aestivum*) species of wheat. However, elevated CO_2 brought about greater photosynthesis in tetraploid and hexaploid compared to diploid. (Table 2)

The stomatal conductance was higher in diploid followed by tetraploid and lowest in hexaploid wheats. Elevated CO_2 decreased the stomatal conductance in all the species. The reduction was more in diploid (33 %) and tetraploid (35 %) compared to hexaploid (9.1 %) wheat. The chlorophyll content was more in hexaploid compared to diploid and tetraploid wheat leaves. Elevated CO_2 brought about marked reduction in the total chlorophyll content of leaves. The reduction was greater in tetraploid and hexaploid compared to diploid wheat leaves. The leaf area of hexaploid wheat was more than diploid and tetraploid wheat irrespective of treatment. Elevated CO_2 brought about significant increase in the leaf area of all the wheat species. The increase was greater in hexaploid (21.8 %) and tetraploid (18.7 %) compared to diploid (7.5 %). (Table.2)

The total sugar content in leaves was more in diploid followed by tetraploid and minimum in hexaploid. Elevated CO_2 increased the total and reducing sugars maximum in diploid compared to tetraploid and hexaploid wheats. The nitrogen content in diploid and tetraploid wheat leaves was higher than that of hexaploid. Elevated CO_2 markedly reduced the nitrogen content in leaves of all the species of wheat. The interaction species X CO_2 treatment was not significant. (Table 2)

Amylase activity was greater in hexaploid compared to diploid and tetraploid wheat species. Elevated CO_2, treatment brought about reduction in amylase activity in the seeds. The interaction treatment X species was not significant.

Total protein from leaf samples at flowering and from seeds at their development and harvest stages were analysed on SDS page. Protein bands of these samples showed qualitative and quantitative differences at flowering and grain filling stages. The proteins with molecular weight 110, 85, 73, 58, 46, 39, 35, 31, 19 and 15 KDa were present in all the wheat species. However, protein bands of 110, 85, 73, 35, 31, 30, and 15 KDa were of low intensity in all the samples. Protein bands of 58, 46, 39, and 14 KDa were more intense in tetraploid wheat species. Proteins of 64 and 42 KDa molecular weight were present in diploid wheat species.They were absent in tetraploid and hexaploids, whereas, 32 KDa protein was observed in tetraploid and hexaploid wheats. The protein with 38 KDa molecular weight was observed in elevated CO_2 treated hexaploid wheat.



*indicates significant difference at $p<0.05$

Fig. 3. Effect of elevated CO_2 on the size of grain in wheat species

Table 3. Effect of increased CO_2 conditions on amylase activity (µg maltose released per µg protein) (average±SE) of the harvested seeds of diploid, tetraploid and hexaploid wheat Species.

Wheat species	CO_2 Condition	Amylase (seed 0h)
Diploid	Ambient	115.68±0.85
	Elevated	110.71±4.18
Tetraploid	Ambient	98.771±3.19
	Elevated	94.291±6.16
Hexaploid	Ambient	126.231±3.91
	Elevated	122.591±0.69
CD at 5 % P CO_2 Cond.		2.85
Wheat species		8.05
CO_2 Cond. × Wheat species		N.S.

The protein banding pattern of SDS-PAGE for 1st stage of grain development ranged between 100-58 KDa molecular weight. Proteins of 99, 98, 85 and 58 KDa molecular weight were present in all species irrespective of the treatment. Protein bands of 100 and 70 KDa molecular weights were observed in tetraploid and hexaploid wheats in both ambient and elevated CO_2 conditions whereas, 95 KDa protein band was observed only in diploid and tetraploid wheat species under ambient condition. At later stage of grain filling, proteins of 85, 70, and 46 KDa were present in the wheat species intensely irrespective of treatment. However, 33 KDa protein band was found only in diploid wheat under elevated CO_2 condition (Fig.3). This is the preliminary analysis of SDS PAGE. An attempt to correlate the protein banding pattern with grain quality is under process.

The dry weight of whole plant was significantly more in hexaploid followed by tetraploid and minimum in diploid wheat. The elevated CO_2 brought about significant increase in the dry weight of whole plant in all the species. The increase was significantly greater in hexaploid (46 %) and tetraploid (26 %) compared to diploid wheats (21 %). (Table.4 and Fig.3)

The grain yield in hexaploid species was significantly greater followed by tetraploid and minimum in diploid irrespective of the treatment. Elevated CO_2 brought about significant increase in the grain yield by 22 % in hexaploid, 16 % in tetraploid and 13 % in diploid wheats. The 1000-grain weight was significantly more in hexaploid wheat (28 g) followed by tetraploid (25 g) and minimum in diploid (17g). Neither the effect of CO_2 nor the interaction species X CO_2 treatment were significant for this character. Dry weight at harvest also showed similar trend as that observed at anthesis, however, the interaction treatment X species was not significant for this stage. The harvest index was significantly greater in hexaploid (31 %) compared to tetraploid (27 %) and diploid (7.6 %). Elevated CO_2 brought about significant increase in the harvest index varying as 15 % in hexaploid, 11% in tetraploid and 6% in diploid wheats. (Table 2).

The harvested seeds of diploid (*Triticum monococcum*) tetraploid (*Triticum durum*) hexaploid (*Triticum aestivum*) were examined for their grain size. The length of grain was significantly greater in tetraploid followed by diploid and hexaploid, however, the width of grain was higher in hexaploid compared to diploid and tetraploid. The elevated CO_2 significantly increased the length & width of wheat grains. The elevated CO_2 induced increase in the length and width of hexaploid wheat was significant, whereas, such an increase in tetraploid and diploid wheats was not significant (Table. 4).

DISCUSSION

Significant genotype variation exists among different wheat species with regard to their photosynthetic efficiency. The diploid wheat *Triticum monococcum* possess photosynthetic rate considerably higher than the hexaploid wheat *Triticum aestivum*. Although higher rate of photosynthesis has been associated with smaller

Table 4. Effect of elevated CO_2 on the length and breadth of seeds of diploid, tetraploid and hexaploid wheat species.

Wheat species	CO_2 concent-ration	Length (microns)	Breadth (microns)
Diploid	Ambient	5989.35±156.84	2485.73±21.44
	Elevated	6180.06±74.29	2507.55±30.23
Tetraploid	Ambient	7785.39±183.54	2192.59±80.61
	Elevated	7794.36±107.46	2306.26±36.53
Hexaploid	Ambient	5181.04±53.92	2532.95±37.05
	Elevated	5680.78±62.33	2863.80±90.49
CD at 5 % CO_2 Conc.		191.54	91.55
Wheat species		234.58	112.13
CO_2 Cond. × Wheat species		331.75	158.57

leaf size linked with A genome in wild diploid wheat, addition of D genome to hexaploid wheat (ABD) has changed the expression of various characters as well as altered the gene expression of A and B genomes in *Triticum aestivum* (Uprety *et al*,1987 and Bansal *et al* 1993). Genetic improvements and environmental changes have resulted in larger grain size and yield in modern species of *Triticum aestivum* compared to primitive *Triticum monococcum.* Dunston *et al* (1973) attributed it to lower CO_2 compensation point and low photorespiratory activity in diploid compared to hexaploid. Dean and Leech (1982) gave an alternate explanation that the effective concentration of CO_2 is higher in the stroma of diploid and tetraploid compared to hexaploid wheat.

Present study demonstrated variable response of diploid, tetraploid and hexaploid wheat species to the elevated CO_2. Two direct effects can explain the observed responses of different species of wheat to elevated CO_2 (Cure and Acock, 1986): 1. Increased photosynthesis and decreased photorespiration, 2. Decreased stomatal conductance. Effect of elevated CO_2 on biomass production, grain yield and developmental aspects are explicable as consequence of these. Study demonstrated that the modern cultivar *Triticum aestivum* wheat is still largely source limited. It appeared to have less assimilates than diploid wheat. It was observed that while grain and leaf size increased more or less in parallel with the increase in ploidy from diploid to hexaploid form but the photosynthetic rate per unit area has fallen markedly. It was also seen that total chlorophyll content increased with the level of ploidy. Austin *et al* (1986) also similarly reported increase in chlorophyll content with increase in ploidy level, however, in the present study the genetic basis of this variation was not analyzed**.** The diploids had smaller leaves with higher N content and the hexaploid wheat had larger leaves with low nitrogen content. Similarly the yield was greater in hexaploid with higher harvest index compared to diploid with low harvest index.

Presence of higher amylase activity in hexaploid wheats indicated the evolution of better carbohydrate digestibility present in grains compared to other wheat species. However, the CO_2 induced reduction in amylase may possibly be due to reduction of nitrogen status of the leaves and seeds. The presence of dense band in low molecular weight proteins in tetraploid wheat under elevated CO_2 may be related to its insensitivity to elevated CO_2 response. Whereas, less effect of elevated CO_2 on the intensity of these low molecular proteins brought about significant responsiveness of diploid and hexaploid wheats to elevated CO_2. The presence of 38 KDa proteins under elevated CO_2 in hexaploid could possibly be responsible to their better responsiveness to the high CO_2 concentration.

Although the rate of photosynthesis and production of sugars was greater in diploids but due to their poor sink development, photosynthate produced by them could not be utilized for the development of yield. Subsequently the photosynthesis rate of hexaploid wheat was considerably lower with greater sink capacity and the available source could not produce that much photosynthate to cater the demand of their sink potential. Thus elevated CO_2 added higher amount of photosynthates to the hexaploids resulting more grain yield and greater harvest index. It was also seen that the greater nitrogen content present in diploids could not be utilized due to their poor sink capacity. Whereas, in hexaploid wheats the greater size of photosynthetic area (Larger size of leaf) could produce more photosynthates under elevated CO_2 condition. The larger leaf area and greater grain weight caused by elevated CO_2 had additive effect in improving the productivity of hexaploid wheats, whereas, such a source sink balance was not present in diploid wheats. The variation in the effect of CO_2 may be an indirect consequence of differences in phenology and sink size since the effect of CO_2 and temperature also reduced the duration of growth and development (Sujatha *et al*, 2008). The increasing CO_2 may present opportunities to breeders and possibly allow them to select for allocation of assimilates away from leaves to roots and seeds and make technological adjustments in wheat productivity by breeding, incorporating new genetic resources and by altering agronomic techniques.

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