BRIEF COMMUNICATION

Effects of elevated CO_2 and nitrogen on wheat growth and photosynthesis

M. PAL[1], L.S. RAO, V. JAIN, A.C. SRIVASTAVA*, R. PANDEY, A. RAJ and K.P. SINGH

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

Abstract

The effects of nitrogen [75 and 150 kg (N) ha^{-1}] and elevated CO_2 on growth, photosynthetic rate, contents of soluble leaf proteins and activities of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and nitrate reductase (NR) were studied on wheat (*Triticum aestivum* L. cv. HD-2285) grown in open top chambers under either ambient (AC) or elevated (EC) CO_2 concentration (350 ± 50, 600 ± 50 µmol mol^{-1}) and analyzed at 40, 60 and 90 d after sowing. Plants grown under EC showed greater photosynthetic rate and were taller and attained greater leaf area along with higher total plant dry mass at all growth stages than those grown under AC. Total soluble and Rubisco protein contents decreased under EC but the activation of Rubisco was higher at EC with higher N supply. Nitrogen increased the NR activity whereas EC reduced it. Thus, EC causes increased growth and P_N ability per unit uptake of N in wheat plants, even if N is limiting.

Additional key words: nitrate reductase, protein content, Rubisco, specific leaf area, *Triticum aestivum*.

The atmospheric CO_2 concentration has been increasing from pre-industrial period and a two-fold increase has been predicted over the next 25 years (Houghton *et al.* 1996). This increased atmospheric CO_2 concentration is likely to influence growth, development and productivity of crop plants (Makino *et al.* 2000). Many studies have reported reductions in plant N concentration due to elevated CO_2, which can increase the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco, EC 4.1.1.39) efficiency and cause mobilization of nitrogen (Makino *et al.* 2000, Uprety and Mahalaxmi 2000). Plants with increased Rubisco efficiency under EC could require less N for their biomass production (Sage *et al.* 1988) but the changes in activity of nitrate reductase (NR, EC 1.6.6.1), a key enzyme involved in N assimilation in plants, under CO_2 enriched atmosphere is poorly known. There are indications of a decreased NR activity due to higher CO_2 levels (Ferrario-Mery *et al.* 1997, Geiger *et al.* 1998, Pal *et al.* 2004) whereas more N resulted in increased NR activity (Cruz *et al.* 2004). The interactions between elevated CO_2 and variable nitrogensupplies have been studied on rice (Aben *et al.* 1999, Moynul Hague *et al*. 2005) and *Brassica* (Uprety and Mahalaxmi 2000). The present experiment attempted to study the effects of elevated CO_2 and nitrogen doses on growth and photosynthetic characteristics of wheat. In addition, activities of key enzymes, Rubisco and NR, involved in photosynthesis and N-metabolism were also investigated.

Wheat plants (*Triticum aestivum* L. cv HD-2285) were grown in earthen pots containing sandy loam soil, in the open top chambers at the Plant Physiology Division, Indian Agricultural Research Institute, New Delhi. Recommended doses of nitrogen [normal-150 kg ha^{-1} (N_{150}), low-75 kg ha^{-1} (N_{75})] were applied in three splits. Modified naturally lit open top chambers (OTC) lined with 120 µm thick polyvinyl chloride sheet, as described by Rogers *et al.* (1983) were designed to expose the plants to elevated CO_2. The height and diameter of the OTC were 1.8 m and 1.6 m, respectively. One set of plants was grown under ambient (AC, 350 ± 50 µmol mol^{-1}), the other group under elevated CO_2 (EC, 600 ± 50 µmol mol^{-1}). There were five replicated chambers each for

Received 27 September 2004, *accepted* 2 February 2005.

Abbreviations: AC - ambient CO_2; EC - elevated CO_2; NR - nitrate reductase; OTC - open top chamber; P_N - net photosynthetic rate; Rubisco - ribulose-1,5-bisphosphate carboxylase/oxygenase; SLA - specific leaf area.

**Present address*: Division of Biochemistry & Plant Physiology, Sher-e-Kashmir University of Agricultural Sciences & Technology, R.S. Pura, Jammu, India

[1] Corresponding author; fax: (+91) 11 2573 8766, e-mail: madanpal@yahoo.com

ambient and elevated CO_2. The concentration of CO_2 inside the chambers was measured regularly using portable photosynthetic system *LI-6200,* (*LICOR*, Lincoln, NE, USA) and was monitored by a flow meter as described by Pal *et al.* (2004).

Five plants selected at random, from each chamber were harvested at 40, 60 and 90 d corresponding to vegetative, flowering and reproductive stages and separated into leaf, stem and root for growth analysis. Total leaf area per plant was measured using a leaf area meter (*LI-3000, LICOR*). Specific leaf area (SLA) was calculated by using the formula suggested by Gardner *et al.* (1985). The rate of photosynthesis was measured at 40, 60, and 90 d after sowing using *LI-6200* when the photosynthetically active radiation (PAR) ranged between 1000 - 1350 μmol m^{-2} s^{-1}. For all physiological and biochemical analysis, the top-most fully expanded leaf of the main culm was used.

In vivo NR activity was assayed in the leaves following the method of Nair and Abrol (1973). Carboxylase activity was determined by radiochemical method according to Servaites *et al.* (1984). Initial activity was measured at 25 °C by injecting 0.05 cm^3 of 5 mM RuBP and 0.025 cm^3 of soluble leaf extract into the assay mixture containing 50 mM Tris-HCl (pH 8.0), 20 mM $MgCl_2$, 0.1 % (m/v) bovine serum albumin, 10 mM $NaHCO_3$ (74 kBq per assay) in a total volume of 0.5 cm^3. The reaction was terminated after 60 s by adding 0.1 cm^3 of 6 M acetic acid. The material was dried at 65 °C and acid stable ^{14}C was estimated by liquid scintillation counter (Packard *TRICARB*, *Model 1600TR*, Meridian, USA). The total activity was determined in a similar manner with the exception that leaf extract and assay mixture were incubated at 25 °C for 10 min and then 0.05 cm^3 of 5 M RuBP was added. From initial and total activities, the percent activation of the enzyme was calculated. The amount of Rubisco in the crude extract was estimated by polyacrylamide gel electrophoresis following the procedure of Makino *et al*. (1986). The total soluble proteins were estimated by Bradford's method (Bradford 1976). The experiment was laid out in completely randomized block design with five replications for each treatment. Statistical analysis of the data was done following the method of analysis of variance (*ANOVA*) (Panse and Sukhatme 1967). The critical difference (CD) was calculated at 5 % probability level.

The higher N supply and EC had a positive influence on plant height, shoot, root and total plant biomass production (Table 1). EC/N_{150} resulted in 90 % and 73 % increment in height at 40 and 60 d, respectively compared to the AC/N_{75}. At the vegetative stage, plants were highly benefited with higher CO_2 and N_{150} levels. The shoot dry mass was 2.1-folds higher at EC/N_{150} than that at EC/N_{75}. Similarly, maximum root dry mass was recorded under higher level of N and EC. The total plant biomass was also increased under EC and with higher N level. Our earlier report on tissue N concentration under EC confirms that the additional N dose compensates for its deficiency and promotes plant growth in wheat (Pal *et al.* 2003). Similar increase in plant height, root and shoot dry mass, and total plant biomass due to exposure to EC has been reported (Rogers *et al*. 1996, Ziska *et al.* 1996, Uprety and Mahalaxmi 2000). Increase in root biomass indicates that under EC, relatively more assimilates were partitioned towards roots. Earlier findings also indicated relatively greater partitioning of carbon to the roots due to high CO_2 (Bazzaz 1990). Similar increase in root biomass due to increase in N supply under EC has been reported in rice (Ziska *et al.* 1996). These reports suggest that the effect of EC is more when there is optimum supply of all the nutrient elements.

Table 1. Effects of ambient (AC) and elevated (EC) CO_2 and two nitrogen levels (N_{75}, N_{150}) on growth parameters of wheat at different growth stages. Values are means ± SE, n = 5; N_{75} = low nitrogen dose (75 kg N ha^{-1}); N_{150} = normal nitrogen dose (150 kg N ha^{-1}).

Treatments		Plant height [cm]	Shoot d.m. [g $plant^{-1}$]	Root d.m. [g $plant^{-1}$]	Total plant d.m. [g $plant^{-1}$]	Leaf area [cm^2 $plant^{-1}$]	Leaf area/mass [cm^2 g^{-1}]
40 d	N_{75} AC	21.33 ± 2.38	0.29 ± 0.02	0.09 ± 0.01	0.38 ± 0.02	96.11 ± 6.42	282.68 ± 6.04
	N_{75} EC	29.90 ± 2.22	0.59 ± 0.04	0.17 ± 0.01	0.76 ± 0.02	124.70 ± 5.30	265.32 ± 7.15
	N_{150} AC	26.47 ± 3.55	0.57 ± 0.04	0.10 ± 0.01	0.67 ± 0.03	134.73 ± 8.89	224.55 ± 6.42
	N_{150} EC	40.53 ± 1.89	1.24 ± 0.07	0.19 ± 0.01	1.43 ± 0.06	195.35 ± 11.0	191.52 ± 4.28
60 d	N_{75} AC	32.83 ± 3.33	0.83 ± 0.04	0.19 ± 0.01	1.02 ± 0.04	183.16 ± 13.56	281.78 ± 5.92
	N_{75} EC	44.60 ± 2.05	1.88 ± 0.10	0.44 ± 0.06	2.32 ± 0.08	265.15 ± 10.31	315.65 ± 7.85
	N_{150} AC	37.87 ± 2.89	1.14 ± 0.12	0.42 ± 0.07	1.56 ± 0.05	274.65 ± 16.03	280.26 ± 6.60
	N_{150} EC	56.73 ± 3.31	2.78 ± 0.24	1.04 ± 0.09	3.82 ± 0.09	380.23 ± 14.78	447.33 ± 8.44
90 d	N_{75} AC	76.67 ± 2.84	1.61 ± 0.11	0.17 ± 0.07	1.78 ± 0.05	101.76 ± 10.20	128.81 ± 3.78
	N_{75} EC	87.67 ± 4.80	3.02 ± 0.18	0.39 ± 0.04	3.41 ± 0.07	125.00 ± 9.88	127.55 ± 3.16
	N_{150} AC	82.33 ± 3.39	2.24 ± 0.29	0.85 ± 0.08	3.09 ± 0.07	191.35 ± 12.35	140.70 ± 4.05
	N_{150} EC	95.00 ± 4.66	4.39 ± 0.31	1.89 ± 0.05	6.28 ± 0.24	264.01 ± 15.61	145.86 ± 3.90

Table 2. Effects of ambient (AC) and elevated (EC) CO_2 and two nitrogen levels (N_{75}, N_{150}) on net photosynthetic rate, leaf soluble protein, and Rubisco content and activities of nitrate reductase and Rubisco of wheat at different growth stages. Values are means ± SE, *n* = 5; N_{75}= low nitrogen dose (75 kg N ha^{-1}); N_{150}= normal nitrogen dose (150 kg N ha^{-1}).

Treatments		Photosynthetic rate [μmol m^{-2} s^{-1}]	Protein content [μg g^{-1}(f.m.)]	Rubisco content . [μg g^{-1}(f.m.)]	Nitrate reductase [μmol g^{-1}(f.m.) h^{-1}]	Rubisco activity [μmol(CO_2) g^{-1}(f.m.) s^{-1}] total	initial	[%]
40 d	N_{75} AC	13.10 ±0.41	1516 ± 28.3	708 ± 16.3	808.9 ± 23.59	15.45 ± 0.26	8.12 ± 0.20	53.0 ± 1.00
	N_{75} EC	20.15 ± 0.56	1380 ± 31.0	640 ± 12.2	724.5 ± 18.03	17.30 ± 0.33	10.57 ± 0.21	60.0 ± 1.07
	N_{150} AC	15.04 ± 0.33	1650 ± 39.4	782 ± 14.9	933.3 ± 21.52	19.30 ± 0.41	11.05 ± 0.17	57.0 ± 1.01
	N_{150} EC	25.51 ± 0.61	1544 ± 32.7	712 ± 17.3	902.2 ± 23.10	21.55 ± 0.38	15.86 ± 0.29	74.0 ± 1.05
60 d	N_{75} AC	14.98 ± 0.47	1560 ± 34.2	804 ± 20.1	401.3 ± 9.62	16.68 ± 0.37	8.94 ± 0.17	54.0 ± 1.03
	N_{75} EC	22.40 ± 0.41	1528 ± 27.1	762 ± 12.0	292.4 ± 7.88	17.24 ± 0.30	10.30 ± 0.24	60.0 ± 1.00
	N_{150} AC	15.16 ± 0.40	1644 ± 40.1	1036 ± 23.5	448.0 ± 11.91	18.78 ± 0.32	11.14 ± 0.34	59.0 ± 1.12
	N_{150} EC	23.78 ± 0.48	1590 ± 35.5	954 ± 16.4	360.9 ± 7.56	20.07 ± 0.44	14.72 ± 0.37	73.0 ± 1.04
90 d	N_{75} AC	15.01 ± 0.38	1584 ± 35.7	978 ± 17.1	429.3 ± 11.4	15.80 ± 0.24	8.25 ± 0.14	52.0 ± 1.05
	N_{75} EC	18.62 ± 0.43	1518 ± 31.4	944 ± 22.6	388.9 ± 9.26	17.66 ± 0.30	11.02 ± 0.21	62.0 ± 0.97
	N_{150} AC	16.00 ± 0.31	1738 ± 31.6	1294 ± 27.9	522.7 ± 10.27	18.96 ± 0.41	11.07 ± 0.29	58.0 ± 0.99
	N_{150} EC	22.28 ± 0.40	1650 ± 40.3	1178 ± 24.6	365.0 ± 7.81	22.85 ± 1.49	16.42 ± 0.31	72.0 ± 1.12

The effect of elevated CO_2 on leaf area was more significant when plants were grown with N_{150} over N_{75}. At EC/N_{150}, the increase in leaf area was 57, 43 and 111 % compared to EC/N_{75} at 40, 60 and 90 d, respectively. Among the growth stages, maximum (159 %) increase in leaf area was observed at 90 d under EC/N_{150} compared to AC/N_{75}. Further, plants grown at EC showed a significant decrease in SLA over AC grown plants at 40 and 90 d whereas at 60 d, SLA was increased to 12 % at N_{75} and 60 % at N_{150}. Similar increase in leaf area per plant has been reported in rice (Ziska *et al.* 1996) and *Brassica* (Uprety and Mahalaxmi 2000) with the increase in dose of N under EC. Furthermore, EC significantly reduced SLA at 40 d and 60 d of plant growth, which suggests an increase in leaf thickness and greater tissue density in the leaves. This observation finds support in Rogers *et al.* (1996) and Das (2003) who also found an increased leaf thickness due to higher rate of photosynthesis under EC. Our data also supplements this fact as the P_N was recorded higher under CO_2 enrichment.

Elevated CO_2 markedly increased P_N at both the N levels (Table 2). However, the increase in P_N was maximum at N_{150} compared to N_{75} under EC. The mean increase in P_N over the growth stages was higher due to EC compared to AC grown plants (46 % at N_{75} and 59 % at N_{150}). Moreover, at 60 d, the P_N was increased by 61 % at both the N levels under EC. It was also noted that the P_N of the AC grown plants was constant with time while that of EC grown plants decreased over time. The overall effect was that the increase in P_N caused by EC decreased with time. Various researchers have reported an increase in P_N under EC and variable N levels (Ziska *et al*. 1996, Aben *et al*. 1999, Uprety and Mahalaxmi 2000). In the present study, however, complete acclimation in photosynthesis was not observed but the relative increase in P_N caused by EC decreased with time.

The amount of Rubisco and soluble leaf protein content was lower in EC plants at both the N levels than those grown at AC (Table 2). However, there was an increasing trend in Rubisco content under both AC and EC with time, the maximum increase being noted at 90 d. The plants grown under EC/N_{150} possessed significantly higher activity of Rubisco enzyme ranging between 72 to 74 % at all the growth stages (Table 2). In EC/N_{150} plants, the protein was lower and initial activity was also less compared to AC/N_{150} but the percent activation of the enzyme was very high under EC. In general, at all the three stages of growth, the activation state of Rubisco was higher in plants grown at EC/N_{150}. Reduced protein content in *Brassica* spp was also reported by Das (2003) under to EC. However, in this study, the reduction in Rubisco protein and leaf soluble protein was less under higher N level with CO_2 enrichment as compared to low N dose. Additional N availability is crucial for rapid growth under high CO_2 conditions as a lower N content was recorded in wheat plants growing under EC (Pal *et al.* 2003).

Significant differences were observed in NR activity under EC between stages of growth and nitrogen levels but the interaction between EC and N levels was not significant (Table 2). Highest NR activity was observed during vegetative growth that was almost 2-fold compared to that at 60 and 90 d. However, EC resulted in marked decrease in NR activity at all the growth stages compared to AC grown plants (7, 23 and 21 % at 40, 60 and 90 d, respectively). Application of high dose of N resulted in 16 % mean increase in NR activity. Our report is in agreement with Ferrario-Mery (1997) and Geiger *et al.* (1998) who have also reported a decrease in NR activity at EC. Such reduction in NR activity may be caused either by less availability of NO_3^- substrate or reduction in enzyme protein, which is evident from our data on protein content recorded under EC. Thus, EC causes increased growth and P_N ability per unit uptake of N in wheat plants, even if N is limiting.

References

Aben, S.K., Ghannown, O., Conroy, J.P.: Nitrogen requirements for maximum growth and photosynthesis of rice *Oryza sativa* L. cv. Jarrah grown at 36 and 70 Pa CO_2. - Aust. J. Plant Physiol. **26**: 759-766, 1999.

Bazzaz, F.A.: The response of natural ecosystems to the rising CO_2 levels. - Annu. Rev. Ecol. Syst. **21**: 167-96, 1990.

Bradford, M.M.: A rapid and sensitive method for quantification of protein utilizing the principal of protein dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Cruz, J.L., Mosquim, P.R., Pelacani, C.R., Araujo, W.L., DaMatta, F.M.: Effects of nitrate nutrition on nitrogen metabolism in cassava. - Biol. Plant. **48**: 67-72, 2004.

Das, R.: Characterization of response of *Brassica* cultivars to elevated carbon dioxide under moisture stress. - Ph. D. Thesis. Indian Agricultural Research Institute, New Delhi 2003.

Ferrario-Mery, S., Thibaud, M.C., Betsche, T., Valadier, M.H. Foyer, C.H.: Modulation of carbon and nitrogen metabolism and of nitrate reductase, in untransformed *Nicotiana plumbaginifolia* during CO_2 enrichment of plants grown in pots and hydroponic culture. - Planta **202**: 510-521, 1997.

Gardner, F.P., Pearce, R.B., Mitchell, R.L.: Physiology of Crop Plants. - The Iowa State University Press, Ames 1985.

Geiger, M., Walch-Piu, L., Harnecker, J., Schulze, E.D., Ludewig, F., Sonnewald, U., Scheible, W.R., Stitt, M.: Enhanced carbon dioxide leads to a modified diurnal rhythm of nitrate reductase activity in older plants and a large stimulation of nitrate reductase activity and higher levels of amino acids in higher plants. - Plant Cell Environ. **21**: 253-268, 1998.

Houghton, J.T., Miera Filho, L.G., Callander, B.A., Harris, N., Kattenberg, A. K., Maskell, K. (ed.): Climate Change 1995: The Science of Climate Change. - Cambridge University Press, Cambridge 1996.

Makino, A., Mae, T., Ohira, K.: Colorimetric measurement of protein stained with Coomassie Brillient Blue R on sodium dodecyl sulfate polyacrylamide gel electrophoresis by eluting with formamide. - Agr. biol. Chem. **50**: 1911-1912, 1986.

Makino, A., Nakano, H., Mae, T., Shimada, T., Yamamoto, N.: Photosynthesis, plant growth and N allocation in transgenic rice plants with decreased Rubisco under CO_2 enrichment. - J. exp. Bot. **51**: 383-389, 2000.

Moynul Haque, M., Hamid, A., Khanam, M., Biswas, D.K., Karim, M.A., Khaliq, Q.A., Hossain, M.A., Uprety, D.C.: Elevated CO_2 effect on leaf chlorophyll and nitrogen contents in rice during post-flowering phases. - Biol. Plant. **49**: in press, 2005.

Nair, T.V.R., Abrol, Y.P.: Nitrate reductase activity in developing wheat ears. - Experientia **29**: 480-481, 1973.

Pal, M., Kartikeyapandian, V., Jain, V., Srivastava, A.C., Raj, A., Sengupta, U.K.: Biomass production and nutritional levels of berseem (*Trifolium alexandrium*) grown under elevated CO_2. - Agr. Ecosyst. Environ. **101**: 31-38, 2004.

Pal, M., Rao, L.S., Srivastava, A.C., Jain, V., Sengupta, U.K.: Impact of CO_2 enrichment and variable nitrogen supplies on composition and partitioning of essential nutrients of wheat. - Biol. Plant. **47**: 227-231, 2003.

Panse, V.G., Sukhatme, P.T. (ed.): Statistical Methods for Agricultural Workers. - Indian Council of Agricultural Research, New Delhi 1967.

Rogers, G.S., Milham, P.J., Gillings, M., Conroy, J.P.: Sink strength may be the key to growth and nitrogen responses in N-deficient wheat at elevated CO_2. - Aust. J. Plant Physiol. **23**: 253-264, 1996.

Rogers, H.H., Heck, W.W., Heagle, A.S.: A field technique for the study of plant responses to elevated CO_2 concentration. - Air Pollut. Control Assoc. J. **33**: 42-44, 1983.

Sage, R.F., Sharkey, T.D., Seeman, J.R.: The *in vivo* response of the ribulose-1,5-bisphosphate carboxylase activation state and the pool sizes of photosynthetic metabolites to elevated CO_2 in *Phaseolus vulgaris* L. - Planta **174**: 407-416, 1988.

Servaites, J.C., Torisky, R.S., Chao, S.E.: Diurnal changes in ribulose-1,5-biphosphate carboxylase activity and activation state in leaves of field grown soybeans. - Plant Sci. Lett. **35**: 115-121, 1984.

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.

Ziska, L.H., Weerakoon, W., Namuco, O.S., Pamplona, R.: The influence of nitrogen on the elevated carbon dioxide response in field grown rice. - Aust. J. Plant Physiol. **23**: 45-52, 1996.