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NONSTRUCTURAL CARBOHYDRATES AND NITROGEN OF MUNGBEAN GROWN UNDER ELEVATED CARBON DIOXIDE CONCENTRATION

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SUMMARY

This study was conducted to determine the effects of anticipated increase in the levels of atmospheric CO_2 on nonstructural carbohydrates and nitrogen of mungbean *(Vigna radiata* L. Wilczek cv. PS16). Plants were grown in 14' pots inside open top chambers and a CO_2 concentration of 600±50 ppm was maintained from seedling emergence to maturity. Leaflet blades were sampled at 30, 40 and 50 days after germination for the estimation of carbohydrate and nitrogen content. At 30 days, starch, total sugars, non-reducing and reducing sugar content of leaflet blades increased significantly with increasing CO_2 from 365 ppm to 600 ppm but at later stages no significant differences were obtained. Specific leaf weight also increased significantly at 30 days after germination and declined later on. High CO_2 grown leaves of mungbean had lower nitrogen concentration at all growth stages. However, no great difference for nitrogen concentration among treatment at final harvest was found. It suggests that the main effect of CO_2 enrichment on nitrogen metabolism occurred at early vegetative stage.

Key words: **Carbohydrate, CO_2 enrichment, mungbean and nitrogen.**

INTRODUCTION

It is widely accepted that the concentration of atmospheric CO_2 is increasing over the past century and expected to increase even at higher rates due to man made activities. In the recent past, fossil fuel burning, cement manufacture and land use have added CO_2 to the atmosphere at a rate of about 8 Gt. of carbon per year (Post *et al.*, 1990). These human activities are creating problems for global atmosphere and CO_2 concentration has increased from about 280 ppmv in the early 1800's to about 355 ppmv at present. As partial pressure of CO_2 in the global atmosphere is increasing by about one μ bar per year, it is predicted that it will reach 380 bar in 2000 and 534 bar in 2025 (Baes *et al.*, 1977). Therefore, the studies on the effect of increased CO_2 on plant growth and metabolism is of great interest.

Many studies have shown that doubling of CO_2 concentration will increase the carbon exchange rates between plant and the atmosphere (Cure and Acock, 1986; Jarvis, 1989). This is because CO_2 is the primary substrate for photosynthesis and the level of current ambient CO_2 concentration is suboptimal for photosynthesis (Sengupta and Sharma, 1993). The increase in the rate of photosynthesis provides more carbohydrates for plant growth. Earlier experiments clearly showed that raising the concentration of CO_2 above ambient levels increased the plant growth in many C_3 species (Ghannoum *et al.* 1997). The increase in growth appears to be due to partitioning of greater assimilates to the growing organs and accumulation of starch and other non-structural polysaccharides in the leaves (Poorter *et al.*, 1997).

Photosynthetic rate of a plant on the other hand is also influenced by their nitrogen status. Some of the earlier experiments conducted on the effect of elevated CO_2 levels on nitrogen level in plant tissue indicate that CO_2 enrichment lowers the concentration of nitrogen in leaves and alter the distribution pattern of nitrogen within the plant (Hocking and Meyer, 1985; Peet *et al.*, 1986; Lariguaderie *et al.*, 1988). In wheat, it was observed that under elevated CO_2, plants accumulated more nitrogen but the proportional increase in the nitrogen content was unlike to dry matter accumulation (Hocking and Meyer, 1991) but in legumes where plants have nitrogen fixing ability, nitrogen metabolism under high CO_2 will be different because symbiotic nitrogen fixers receive all of their carbon from photosynthates and provide a source of available nitrogen to their host plant. It is therefore, very important to understand the role of biological nitrogen fixation under high CO_2. The objectives of this study were to determine the effects of elevated CO_2 on nonstructural carbohydrates and nitrogen content of leaflet blades during different growth stages of mungbean.

MATERIALS AND METHODS

Mungbean (*Vigna radiata* L. Wilczek cv. PS16) was grown in 14' pots at Plant Physiology Division, Indian Agricultural Research Institute, New Delhi. The soil prepared for pot was of fine quality and thoroughly mixed with sufficient farmyard manure. Five plants in each pot were maintained after thinning. In this experiment, an open top chamber (OTC) was fabricated in our laboratory using pure CO_2 gas to study crop responses to elevated CO_2. The height and diameter of the open top chamber was 1.8 meter and 1.6 meter respectively lined with transparent PVC sheet. Plants were exposed to 600 ± 50 ppm CO_2 from seedling emergence to maturity without much affecting temperature ($\pm 1^\circ$C), RH (± 4) and light intensity ($\pm 4 \mu$E)

Leaves were sampled at 30, 40 and 50 days after germination. Reducing sugar content were estimated by Nelson's arsenomolybdate method (Nelson, 1944) using improved copper reagent of Somogyi (1952). Absorbance was measured at 630 nm in Spectronic-20 (Baush and Lamb, USA). Non-reducing sugar were calculated by subtracting the reducing sugar content from the total sugar content. Dried samples of the residue left after extraction for reducing sugars were used for starch content (Pucher *et al.*, 1950).

Non-protein and protein in form of reduced nitrogen in the trichloro acetic acid (TCA) preserved leaf material was determined by using N-Kjeltech Auto 1030 Analyser, following the procedure detailed in Tecator Manual, 1987.

RESULTS AND DISCUSSION

The specific leaf weight, starch, sucrose, reducing sugar and total sugar content of mungbean leaves increased due to increase in CO_2 concentration at 30 days after germination (Table I, Fig. 1). Starch levels showed the greatest change, with over a 116.88% increase for plants grown at 600 ± 50 ppm CO_2 as compared to the control ($365 \pm$ppm CO_2). For the same CO_2 concentration, non-reducing and reducing sugar levels also increased by 41.18% and 53.05% respectively. Specific leaf weight increased by 78.43%. Increase in starch, reducing and non-reducing sugar contents under elevated CO_2 over

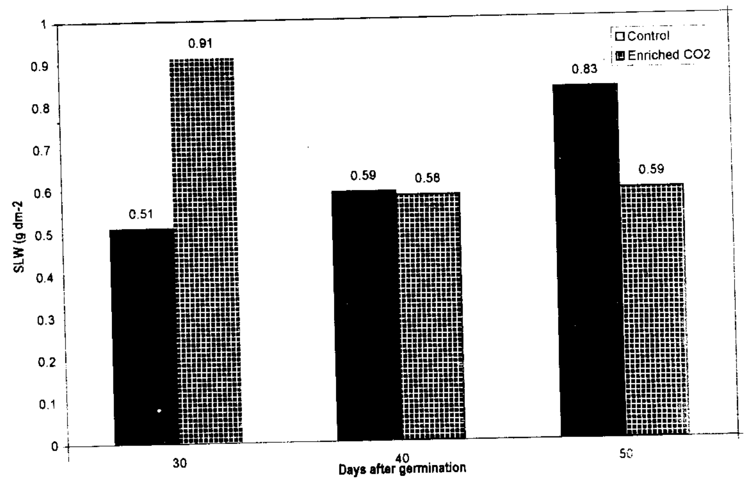

Fig. 1. Effect of elevated CO_2 on specific leaf weight (SLW) at different growth stages

TABLE I. Starch, non reducing sugar, reducing sugar and total soluble sugar in leaves of mungbean grown under elevated CO_2 concentrations. Leaves were sampled from plants at 30, 40 and 50 days after germination.

Days after germination	Starch (mg g^{-1} dry weight)			NRS (mg g^{-1} fresh weight)			RS (mg g^{-1} fresh weight)			TSS (mg g^{-1} fresh weight)		
	C	E	% change	C	E	% change	C	E	% change	C	E	% change
30	182	295	+116.8	1.7	2.4	+41.18	1.79	2.74	+53.07	3.49	5.15	+47.56
40	486	543	+11.79	1.37	2.81	+105.1	2.82	2.85	+1.06	4.19	5.33	+27.21
50	520	571	+9.9	5.05	5.29	+4.75	4.83	5.66	+17.2	9.88	10.95	+10.83

NRS = Non reducing sugar, R = Reducing sugar, TSS = total soluble sugar, C = Control (365 ppm) E = Elevated CO_2 (600±50ppm)

ambient CO_2 plants were narrowed down at 40 and 50 days after germination. Almost equal non-reducing sugar was recorded at 50 days after germination for ambient and elevated CO_2 conditions. Similarly marginal increase in reducing sugar was observed at 50 days after germination under elevated CO_2 grown plants. It is therefore, no significant difference for total sugar was found at 50 days after germination.

There are many reports in other plants which showed that the starch content increases due to long term CO_2 enrichment. Increase in starch content is reported in *Cucumis sativus* (Madore and Godinski, 1985), *Glycine max* (Havelka *et al.*, 1984; Cure *et al.*, 1987) and *Phaseolus sativus* (Hoddinott and Jolliffe, 1988).

In the present investigation, plants grown under high CO_2 maintained a higher level of starch in the leaves throughout the growth period and no evidence of leaf malformation due to high starch accumulation was observed. The major increase in starch content which was observed at 30 days after germination not sufficient to cause inhibition of photosynthesis. Probably there was greater sink demand and greater export takes place during this phase to cause early growth of the plants. Earlier studies on short term CO_2 enrichment in mungbean have shown that starch accumulation occurs due to high CO_2 but the extra carbon fixed due to CO_2 enrichment is exported out of the source leaf within 24 hours (Sharma, 1986). Thus, from this study it appears that the mungbean plants grown under elevated CO_2 (600 ppm) produce greater amount of photosynthates which were partitioned to starch in the source leaf (short distance transport) and also to the growing sink such as shoot and roots (long distance transport). This resulted increase in dry weight of the leaves and stem.

Another important aspect is nitrogen utilization capacity of the plants under high CO_2 conditions. From the above discussion, it is clear that elevated CO_2 caused substantial carbon gain in the plants. The present study showed decreased level of nitrogen when the plants were grown under elevated CO_2. As we know major nitrogen source in plant is protein and in most proteins, nitrogen constitutes 16% of the total makeup. In the present study, protein nitrogen in dry leaves of mungbean harvested at 30, 40 and 50 days after germination was 1.89, 1.18 and 1.17% for ambient CO_2 conditions whereas 1.45, 1.00 and 1.06% for elevated CO_2 condition (Fig. 2). Thus a decrease in protein nitrogen was obtained due to elevated CO_2. Non-protein nitrogen was also lower in elevated CO_2 grown plants at 30 and 40 days after germination. Percentage of total nitrogen in leaves of mungbean under elevated CO_2 conditions was consistently lower than in ambient CO_2 grown plants. Decrease in nitrogen content due to elevated CO_2 was 19.09% at 30 days and 29.59% at 40 days after germination. No significant difference was observed at 50 days after germination. Thus, it appears that the nitrogen taken up by the plants can not substantiate equally to the carbon gain by the plants under elevated CO_2 conditions. In several other C_3 plant species

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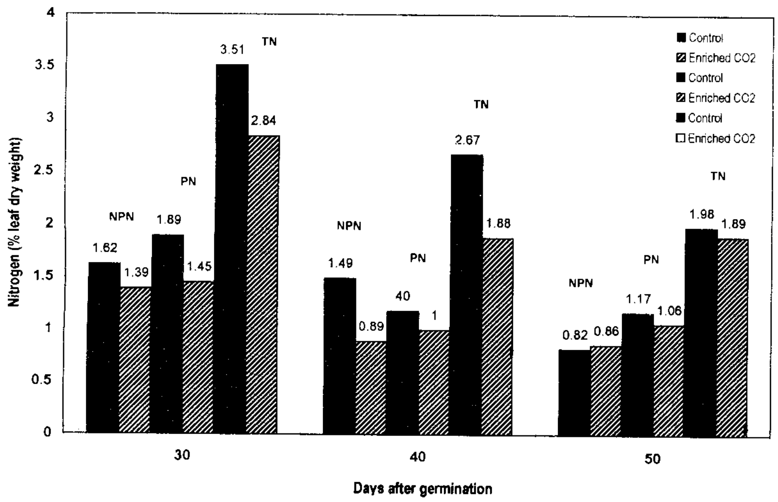

Fig. 2. Effect of elevated CO_2 on non-protein nitrogen (NPN), protein nitrogen (PN) and total nirtogen (TN) in leaves of mungbean at different growth stages

such as wheat (Manderscheid *et al.*, 1995; Billes *et al.*, 1993) and soybean (Allen *et al.*, 1988; Reeves *et al.*, 1994), lowering in nitrogen content was reported. Low nitrogen concentration due to elevated CO_2 is possible due to greater photosynthetic efficiency (Sage *et al.*, 1994). The performance of higher CO_2 grown mungbean with respect to photosynthesis and growth was not inhibited by the reduced nitrogen concentration. It seemed that greater structural leaf weight caused the nitrogen dilution which may be partially compensated by extra layer of mesophyll cells (Vu *et al.*, 1989) thereby preserving the total photosynthetic machinery per unit leaf area (Soussana *et al.*, 1996). It is also possible that a CO_2 induced reduction in total nitrogen concentration may not be due to physiological changes in plant nitrogen use efficiency, but is probably a size dependent phenomenon resulting from accelerated plant growth.

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REFERENCES

Allen, L. H. Jr., Vu, J.C.V., Valle, R.R., Botte, K.J. and Jones, P.H. (1988). Non structural carbohydrates and nitrogen of soybean grown under CO_2 enrichment. *Crop Sci.*, **28** : 84-94.

Baes, C.F., Goeller, H.E., Olson, J.C. and Rotiy, R.M. (1977). CO_2 and climate: The uncontrolled experiment. *Am Scientist.*, **66** : 310-320.

Billes, G., Rouhier, H. and Bottner, P. (1993). Modification of the carbon and nitrogen allocation in the plant (*T. aestivam* L.) soil system in response to increased atmospheric CO_2 concentration. *Plant and Soil.*, **157** : 215-225.

Cure, J.D. (1986). Crop responses to CO_2 doubling : A literature survey. *Agricultural and forest meteorology.*, **38** : 125-145.

Cure, J.D., Israel, D.W. and Rufty, T.W. (1988). Nitrogen stress effects on growth and yield of nonodulated soybean exposed to elevated carbon dioxide. *Crop Sci.*, **28** : 671-677.

Ghannoum, O., Von. Gaemmerer, S., Barlow, E.W.R. and Conroy, J.P. (1977). The effect of CO_2 enrichment and irradiance on the growth, morphology, and gas exchange of C_3 (*Panicum luxum*) and C_4 (*Panicum antidotale*) grass. *Aust. J. Plant Physiol.*, **24** : 227-237.

Havelka, V.D., Ackerson, R.C., Boyle, M.G. and Wittenbach, V.A. (1984). CO_2 enrichment effects on soybean physiology. I. Effects of long term CO_2 exposure. *Crop Sci.*, **24** : 1146-1149.

Havelka, V.D., Wittenbach, V.A. and Boyle, M.G. (1984b). CO_2 enrichment effects on wheat yield and physiology. *Crop Sci.*, **24** : 1163-1168.

Hocking, P.J. and Meyer, C.P. (1985). Responses of noogorara burr (*Xanthium occidentale* Bertol) to nitrogen supply and CO_2 enrichment. *Ann. Bot.*, **55** : 835-894.

Hocking, P.J. and Meyer, C.P. (1991). Effects of CO_2 enrichment and nitrogen stress on growth, and partitioning of dry matter and nitrogen in wheat and maize. *Aust. J. Plant Physiol.*, **18** : 1339-1356.

Jarvis, P.G. (1989). Atmospheric CO_2 and forests. *Philosophical Transaction of the Royal Society of London.*, **324B** : 369-392.

Larigauderie, A., Hilbert, D.W. and Oechel, W.C. (1988). Effect of CO_2 enrichment and nitrogen availability on resource acquisition and resource allocation in a grass, *Bromus mollis*. *Oecologica.*, **77** : 544-549.

Maderscheid, R., Bender J., Jager, H.J. and Weigel, H.J. (1995). Effects of season long CO_2 enrichment on cereals. II. Nutrient concentration and grain quality. *Agriculture, Ecosystem and Environment.*, **54** : 175-185.

Madore, M. and Grodizinski, B. (1985). Effect of CO_2 enrichment on growth and photoassimilate transport in dwarf cucumber. *J. Plant. Physiol.*, **121** : 59-71.

Mccready, R.M.J., Guggloz, J. and Oven, H.S. (1950). Determination of starch and amylase in vegetable. *Ann. Chem.*, **22** : 1156-1158.

Mckee, I.F. and Woodward, F.I. (1994). CO_2 enrichment responses of wheat : Interactions with temperature, nitrate and phosphate. *New Phytol.*, **127** : 447-453.

Nelson, N. (1944). A photometric adaptation of the Somogyi's method for the determination of glucose. *J. Biol. Chem.*, **153** : 375-380.

Peet, M.M., Huber, S.C. and Patterson, D.T. (1986). Acclimation to high CO_2 in monoecious cucumber. II. Carbon exchange rates, enzyme activities and starch and nutrient concentration. *Plant Physiol.*, **80** : 63-67.

Poorter, H., Berkel, Y.V., Baxter, R., Hertog, J.D., Dijkstra P., Gifford, R.M., Griffin, K.L., Roumet, C., Ray, J. and Wong, S.C. (1997). The effects of elevated CO_2 on the chemical composition and construction costs of leaves of 27 C_3 species. *Plant Cell Environ.*, **20** : 472-482.

Post, W.M., Tsung-Hung, P., William, R.E., Anthony, W.K., Virginia, H.D. and Donald, L.D.A. (1990). The global carbon cycle. *American scientist.*, **78** : 310-326.

Pucher, G.W., Leavenworth, C.S. and Vickery, H.B. (1948). Determination of starch in plant tissues. *Ann. Chem.*, **20** : 850-853.

Reeves, D.W., Rogers, H.H., Prior, S.A., Wood, C.W. and Runion, G.B. (1994). Elevated atmospheric CO_2 effects on sorghum and soybean nutrient status. *J. Plant Nutri.*, **17** : 1939-1954.

Sage, R.F. (1994). Acclimation of photosynthesis to increasing atmospheric CO_2 : The gas exchange perspective. *Photosynth. Res.*, **39** : 351-368.

Sengupta, U.K. and Sharma, A. (1993). CO_2 enrichment effects on photosynthesis and plant growth. In : Eds Abrol YP; Mohanty P and Govindjee Photosynthesis : Photoreactions to plant productivity. Oxford and IBH publishing Co. Pvt. Ltd. New Delhi. pp. 479-508.

Sharma, A. (1986). Studies on relationship of CO_2 assimilation and translocation of assimilates in mungbean. Ph. D. Thesis. P.G. School. IARI. New Delhi.

Somogyi, M. (1952). Notes on sugar determination. *J. Biol. Chem.*, **195** : 19-23.

Soussana, J.F. and Hartwig, U.A. (1996). The effects of elevated CO_2 on symbiotic nitrogen fixation; a link between the carbon and nitrogen cycles in grassland ecosystems. *Plant and Soil.*, **187** : 321-332.

Vu, J.C.V., Allen, L.H.Jr. and Bowes, G. (1989). Leaf ultrastructure, carbohydrates and protein of soybean grown under CO_2 enrichment. *Environ. Exp. Bot.*, **29** : 141-147.