Pak. J. Bot., 43(2): 1127-1131, 2011.

INFLUENCE OF ELEVATED LEVEL OF CO_2 ON BIOCHEMICAL PARAMETERS OF *GLYCINE MAX* (L.) MERR. INOCULATED WITH *BRADYRHIZOBIUM JAPONICUM*

NEELOFER HAMID, AFSHAN REHMAN AND FAIZA JAWAID

Department of Botany, University of Karachi,
Karachi-75270, Pakistan

Abstract

Effect of elevated CO_2 with *Bradyrhizobium japonicum* strain (KUCC-118) on some biochemical parameters of soybean (*Glycine max* (L.) Merr was studied. The sterilized seeds of *Glycine max* were coated with *Bradyrhizobium japonicum*. The seedling of soybean was fumigated with 2% (20000ppm) and 3% (30000ppm) CO_2 for 10 and 20 mins., duration. Seedlings were exposed twice a day in a week to elevated CO_2. The 3% (3000ppm) CO_2 fumigated plants showed weekly reduction in carbohydrate, total protein, total DNA and total RNA as compared to the exposure of 2% (2000ppm) concentration of CO_2. Control plants which were grown in ambient level (330ppm) of CO_2 with inoculation of *Bradyrhizobium japonicum* showed significant increase in biochemical parameters.

Introduction

Glycine max (L.) Merr (soybean) is an important full seasonal cash crop in the world (Berglund & Helms, 2003). It is a good source of low cost protein which is about 32-45%. Seeds of soybean are widely used for multipurpose (Elsheikh *et al*., 2008). For first time sowing in the land, soybean seeds should be inoculated with Rhizobia for getting the high yield of crop, 40-70% of additional carbohydrate and minerals provided by nitrogen enrichment through the supply of soybean rhizobia. Nitrogen is well provided through the nodules of *G. max*. Preavailability of nitrogen in the soil delayed the nodules formation but don't reduce the yield of the crop (Anon., 2002).

Bradyrhizobium japonicum is used as a biofertilizer in the nitrogen deficient soil to increase the productivity of *G. max* (Egamberdiyeva *et al*., 2004). *B. japonicum* has an ability to enhance the growth of *G.max* in that soil where bacteria are not available for nitrogen fixation (Uslu & Esendal, 1998). Inoculation of seeds of *G. max* with *B. japonicum* favours to survive bacteria on the seeds surface before planting. *B. japonicum* infection starts in the root of the *G. max* after the emergences of radical. After 2-3 weeks of infection the process of nodulation is started (Anon., 2002).

Recent studies emphasize that the secure concentration of CO_2 is 350 ppm for the atmosphere of the world. The level of CO_2 in the atmosphere is higher than 350 ppm since 1988. The average concentration of CO_2 in 2009 was 387.35 ppm recorded at Mauna Loa observatory (Anon., 2010). The protein concentration of human plant food in 21^{st} century would be decreased by increasing the CO_2 level. The composition of chemical nutrition in human food is altered by the implication of elevated CO_2 (Taub *et al*., 2007). The net rate of photosynthesis increases by the short term exposure of elevated CO_2 whether the long term exposure of elevated CO_2 declined the net carbon assimilation (Evan, 1985; Hamid *et al*., 2009).

Experiments were therefore carried out on the correlation of elevated CO_2 with *B. japonicum* on physiological and biochemical parameters of *G. max*.

Materials and Methods

The pot experiment was designed to observe the effect of elevated level of CO_2 with combination of *B. japonicum* on *G. max*. Acid washing of sand was done according to Hewitt (1966). Healthy seeds of soybean were sterilized with 1% mercuric chloride then coated with *B. japonicum* by using 4% sucrose solution. Seeds were sown in 15 cm diameter plastic pot which contained 1500 g sand. Ten coated seeds were sown and pots were regularly watered with sterilized distilled water. After 15 days, seedling of soybean were fumigated by 2 (20000ppm) and 3% (30000ppm) concentration of CO_2 in controlled chamber. Different concentrations of CO_2 were supplied in chamber by gas cylinders which were obtained from (The National gas limited Pakistan). Elevated CO_2 enters through the bottom of the chamber through pipe and goes out by the open top. Plants were exposed twice a week for 10 and 20 mins., duration in controlled chamber. Control plants were grown in natural environment ambient level of CO_2. All the treatments were replicated thrice. Biochemical parameters of treatments were examined weekly. The data were recorded individually for statistical analysis by ANOVA technique. The graphic representation of data was possible by "SPSS and SIGMA PLOT" software programs.

Estimation of carbohydrate content: Estimation of carbohydrate was done in plants by Yemm & Willis (1956) method using Anthrone reagent. Absorbance was determined at 620 nm. The carbohydrate content was calculated by µg/mg fresh weight.

Protein estimation: Protein was estimated by Lowry *et al.*, (1951). The optical density was measured at 750 nm against reagent blank. The total protein content was calculated from a standard curve of bovine serum albumin.

Nucleic acid estimation: The total Nucleic acids (DNA & RNA) were investigated by the method of Schmidt & Thannhauser (1945).

Results

Different concentration of CO_2 (20000ppm) and (30000ppm) along with inoculation of *B. japonicum* showed significant ($p<0.001$) decrease in total carbohydrate content of *G. max* leaves as compared to ambient concentration (350ppm) of CO_2 in control plant (Fig. 2). Fumigated plants of 2% (20000ppm) CO_2 showed significant increase ($p<0.001$) in carbohydrate as compared to 3% (30000ppm) concentration of CO_2 (Fig. 2).

Protein relation with respect to week fumigation gradually increased in 2% CO_2 concentration. Fumigation of 2% (20000ppm) CO_2 showed a significant increase ($p<0.001$) in *G. max* leave protein but significantly negative response in 3% (30000ppm) fumigated plants. Activity of rhizobia in ambient level of CO_2 concentration (350ppm) was significantly enhanced ($p<0.001$). Fumigation of CO_2 favor the enhancement of protein in treated plants up to certain level with elevated CO_2. Activity of *B. japonicum* is significantly high ($p<0.001$) in controlled plants (Fig. 2).

Nucleic acids were adversely affected by elevated 2 and 3% CO_2 concentration. It was observed that inoculated plants with 2% CO_2 (20000ppm) fumigation showed ($p<0.001$) an increase whereas inoculated plant with 3% (30000ppm) CO_2 concentration indicates weekly reduction ($p<0.001$) in nucleic acid. Ambient CO_2 with inoculation significantly increased ($p<0.001$) the DNA and RNA content (Fig. 1).

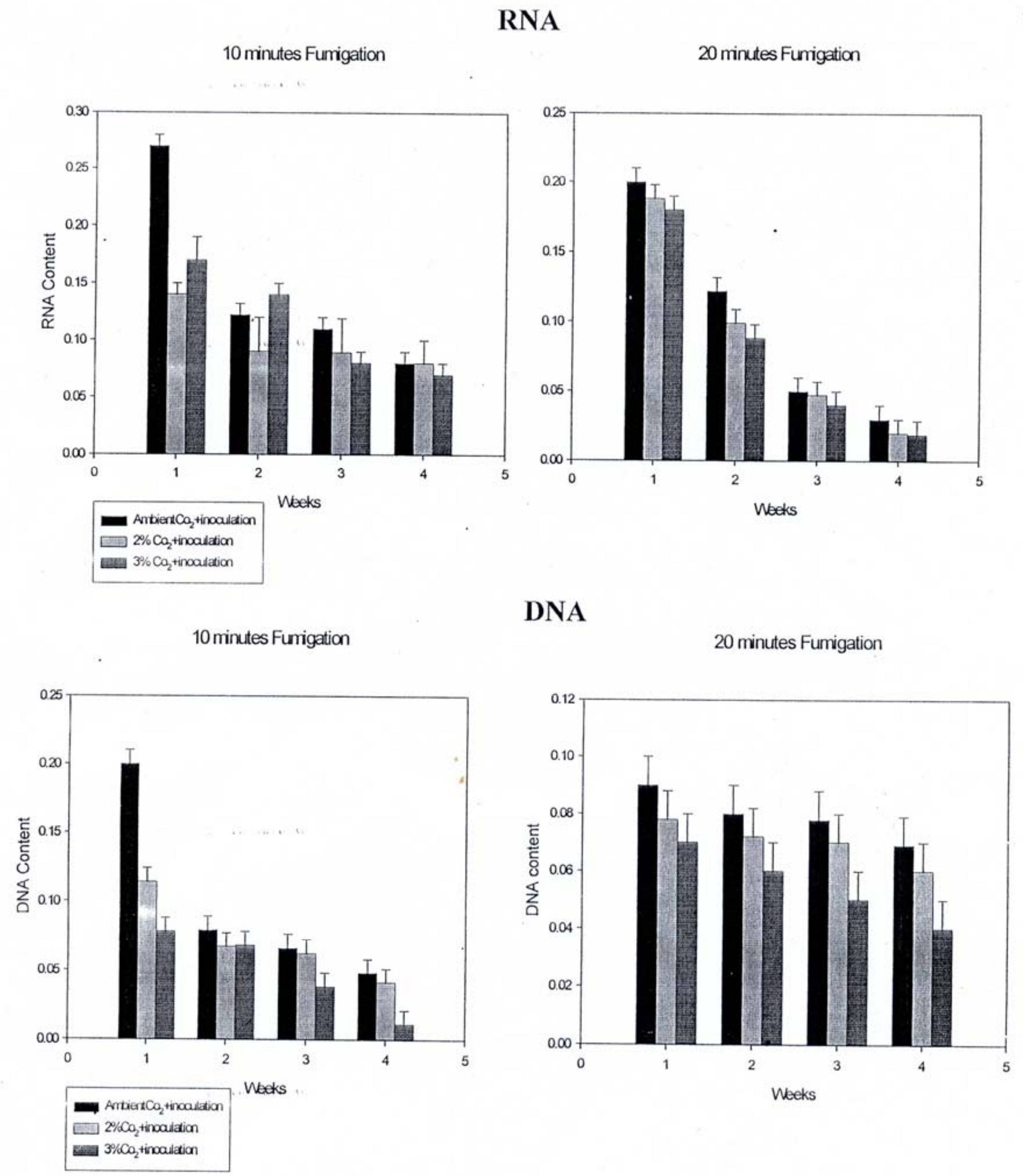

Fig. 1. RNA and DNA content of leaves of uninoculated and inoculated *G. max* at elevated (2000 and 3000ppm) level of CO_2.

Discussion

Carbohydrate was significantly reduced at elevated level of CO_2 in *G. max*. There was reduction in total carbohydrate in *G. max* leaves when exposed to different level of CO_2. The negative result of carbohydrate in 3% CO_2 for 20 min., fumigation (Elsheikh *et al.,* 2008) reported that carbohydrate content in the *G. max* generally decrease with *Rhizobium* inoculation. Fumigated plants with 3% CO_2 indicated that there was a decline in photosynthesis process due to the saturation of CO_2 in leaves that inactivate the reaction centre of leaves. Similarly Tzvetkova & Kolarov (1996) found that the total sugar of stress leaves gradually decreases corresponding with the high stimulation of respiration and inhibition of photosynthesis.

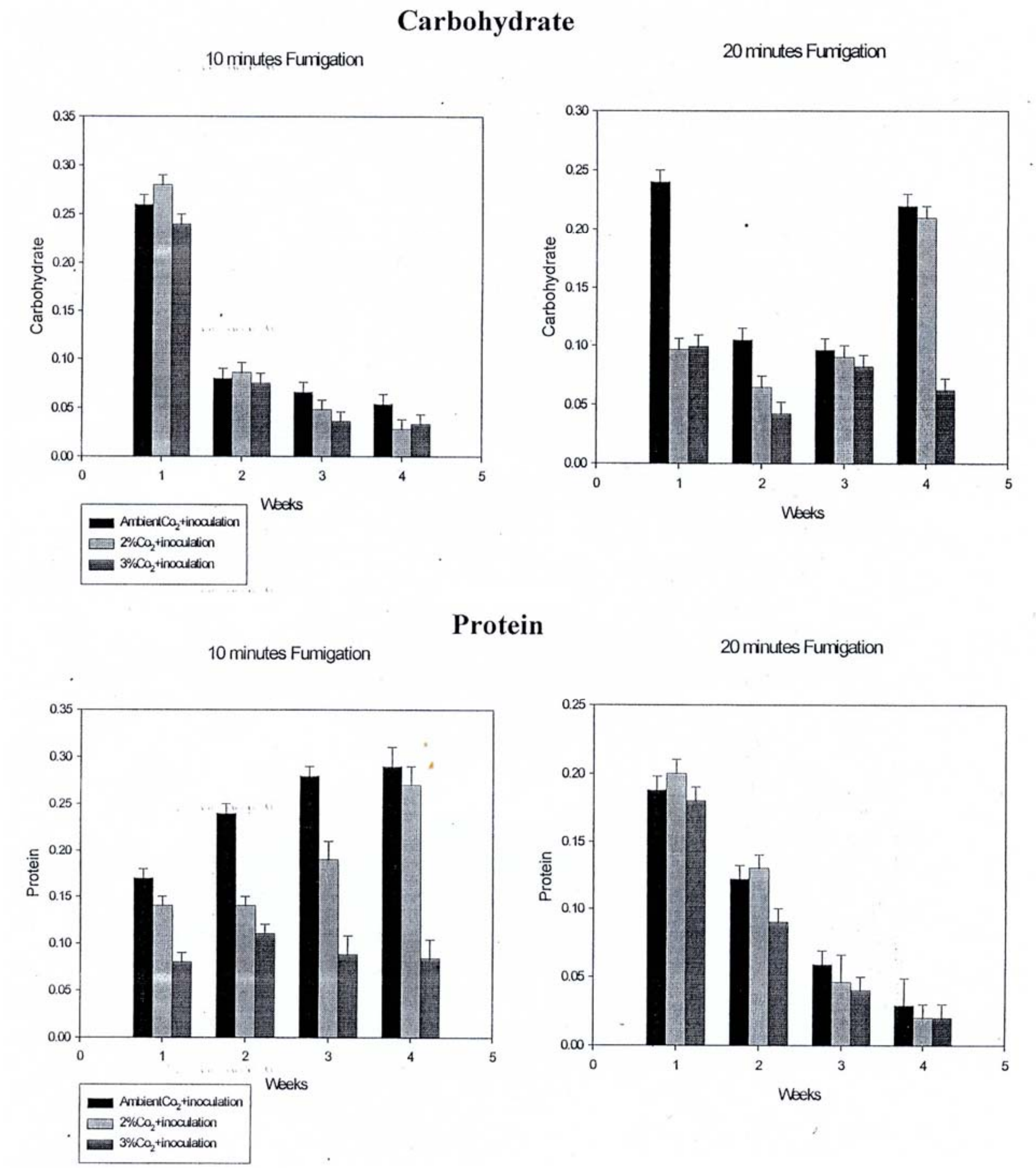

Fig. 2. Carbohydrate and Protein content of leaves of uninoculated and inoculated *G. max* at elevated (2000 and 3000ppm) level of CO_2.

In contrast to effect on the protein content, in ambient level of CO_2 showed a significant increase as compared to 3% CO_2 fumigated plants. Short term exposure (10 mins.,) with 2 and 3% CO_2 indicates a valuable increase but it was not persisting for long term exposure (20 mins.,). It was suggested that the enrichment of CO_2 for long term declined the carbohydrate and diminished the soluble leaves protein by the inactivation of enzymes. It was also described by (Zeiger, 2006; Hamid *et al.*, 2009) that protein content could be decreased by reduction in synthesis and readily utilization of pre- existing protein in far above the ground supply of CO_2.

Decrease in the DNA content is evident in leaves of *G. max* after exposure of elevated level (2000 and 3000ppm). The control plants which were grown in ambient level (330ppm) of CO_2 illustrated the remarkable increase in DNA. 2% CO_2 indicated a significant increase as compared to ambient level of CO_2 but it was significantly decreased in 3% CO_2 fumigated plants. These results were explained by the reports of Jana (1984) and Hamid *et al.*, (2010) in any stress the total nucleic acids were reduced. Thomas & Griffin (1994) concluded the same results that there was a potentially different effect of CO_2 enrichment on *G. max* with short period and long period exposure of elevated CO_2. The long term exposure with inoculation has drastically affected the plant growth and metabolism while the short term exposure have pronounced effects on the plant growth and metabolism.

References

Anonymous. 2010. *Atmospheric CO_2*. Mauna Loa observatory.co$_2$now.org.

Anonymous. 2002. Soybean planting and crop development. Ontario, *Ministry of Agriculture food and rural affairs*. Queens printer for Ontario.

Berglund, R.D. and T.C. Helms. 2003. *Soybean Production*, 2003. NDSU. *www.ag.ndsu.edu.*

Evan, H.D., W.S. Thomas and R.S. Boyd. 1985. Photosynthetic inhibition after long-term exposure of elevated levels of atmospheric carbon dioxide. *Photosynthesis Research*, 7: 175-184.

Elsheikh, A.E.E., S.S.M. Salih, A.A. Elhussein and E.E. Babiker. 2008. Effect of intercropping *Bradyrhizobium* inoculation and chicken manure fertilization on the chemical composition and physical characteristics of soybean seed. *Food Chemistry,* 112(3): 690-694.

Egamberdiyeva, D., D. Qarshieva and K. Davranov. 2004. The use of *Bradyrhizobium* to enhance growth and yield of soybean in calcareous soil in Uzbekistan. *Journal of plant growth regulation,* 23(1): 54-57.

Hamid, N., N. Bukhari and F. Jawaid. 2010. Physiological responses of *Phaseolus vulgaris* to different lead concentration. *Pak. J. Bot.*, 42(1):239-246.

Hamid, N, F. Jawaid and D. Amin. 2009. Effect of short term exposure of two different CO_2 concentrations on growth and some biochemical parameters of physiological responses of edible beans *Vigna radiata* and *Vigna unguiculata*. *Pak. J. Bot.*, 41(4):1831-1836.

Hewitt, E.J. 1966. *Sand and water culture methods used in study of plant.* 2nd ed. Commonwealth Agricultural Bureaux 479-534.

Jana, S and M.A. Choudhuri. 1984. Synergistic effects of heavy metals pollutants on senescence in submerged aquatic plant. *Water, Air and soil Pollution.*, 21(1-4):351-357.

Lowry, O.H., N.J. Rosbrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the folin phenol reagent. *J Biol Chem.,* 193:265-275.

Schmidt, G. and S.J. Thannhauser. 1945. A method for the determination of DNA, RNA and the phosphoproteins in animal tissues. *J Biol Chem.,* 161: 83-89.

Taub, R. D., B. Miller and H. Allen. 2007. Effect of elevated CO_2 on the protein concentration of food crops: a Meta analysis. *Global Change Biology.*, 14(3): 565-575.

Thomas, B.R., and K.L.Griffin. 1994. Direct and indirect effect of atmosphere CO_2 enrichment on leaf respiration of *Glycine max* (L.)Merr. *Plant physiology.,* 104(2): 255-361.

Tzvetkova, N. and D. Kolarov. 1996. Effect of air pollution on carbohydrate and nutrients concentration in some deciduous tree species. *Bulg. J. Plant Physiol*., 221(1-2): 53-63.

Uslu, N. and E. Esendal. 1998. Response to inoculation and sowing date of soybean under Bafra Plain Conditions in the Northern Region of Turkey. *Tr.J.of Agriculture and Forestry,* 22: 525-531.

Yemm, E.W. and A.J. Willis. 1956. The estimation of carbohydrate in the plant extract by anthrone reagent. *J Biol Chem.*, 57: 508-514.

Zeiger, E. 2006. *The effect of air pollution on plants.* Plant Physiology, fourth Edition (On line), Editors Lincoln Taiz and Eduardo Zeiger.

(Received for publication 18 June 2010)