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Physiological and Biochemical responses of two legume species to elevated levels of carbon dioxide

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ABSTRACT
Effects on some selected physiological and biochemical parameters of *Glycine max* and *Phaseolus vulgaris* were inspecting in present investigation using exposure to enhanced CO_2 levels. Enriched CO_2 levels (2 and 3%) were used to exposed two weeks olds *Glycine max* and *Phaseolus vulgaris* seedling for 15 minutes per days, for 40 days duration. Aeration with enriched CO_2 air resulted in higher carbohydrate content as compare to plants grown in ambient CO_2 condition. However, reduction in protein was found under elevated levels of CO_2, the effect was more pronounced at 3% CO_2 level as compared to 2%. The amount of various amino acids was increase in both CO_2 treatments, while decrease was observed in the quantity of glycine and serine. In present study, we furthermore investigate the changes in the activity of peroxidase enzyme, which involved in defense mechanisms of plants under oxidative stress. The activity of peroxidase enzyme was found to be increases, this increased continued till the end of experimental period and higher activity of peroxidase was found in the treated plants than control.

Key words: Amino acid; Carbohydrate; Peroxidase; protein; *Glycine max*; *Phaseolus vulgaris*

1. INTRODUCTION
CO_2 is a natural component of the earth's atmosphere and it is considered as important gaseous nutrient for green plants in order to carry out photosynthesis[1]. The concentration of CO_2 in the air has enhanced slowly since about 1850 from about 280 µl/l to slightly over 340 µl/l at present[2]. The amount of CO_2 that requires by plants to grow vary from plant to plant, it was observed that most of plants will stop growing when the CO_2 level decreases below 150 ppm, 370 ppm was found to be averaged level for normal plant growth, according to another report 1000 to 1400 ppm CO_2 level was found to ideal level for plant growth and it dramatically increase photosynthetic rates and hence the growth[3]. Elevated CO_2 is the primary variable that influences growth, yield and increases aboveground biomass[4].In C_3 crops elevated CO_2 stimulates photosynthesis because ribulose 1, 5-bisphosphate carboxylase/oxygenase (Rubisco) is not CO_2 saturated at current CO_2 level and because elevated level of CO_2 inhibits photorespiration[5]. There has been no significant stimulation of yield was established in C_4 crops because C_4 photosynthesis is saturated under ambient CO_2[6-9].

On other hand CO_2 is consider as major air pollutant[10], because it is an important green house gas and absorbing infrared radiation from the earth and predicated to cause global warming through a process called the green house effect[11]. CO_2 enters the atmosphere through burning of fossil fuels, deforestation and also chemical reactions undergoing in different factories[12]. Many studies concluded that natural phenomena like volcanoes also produced the warming of earth climate[13]. Global warming and increased atmospheric CO_2 is having a major impact on plant distributions; in general, Plants benefit from slightly warmer temperatures and higher CO_2, but not all plants will benefit equally from these conditions, and some may even be harmed[14].

The main intention of this study was to determine the effect of elevated level of CO_2 on total carbohydrate, total protein and individual amino acid content of *Glycine max* and *Phaseolus vulgaris* and to determine the effect of elevated levels of CO_2 exposure on peroxidase activity, which play important role in protecting plants against injury of oxidative stressors. In this study we tested hypothesize that magnitude of responses of both legume species differ with exposure to treatments of CO_2(2 and 3%) and physiological parameters were used to recognize the difference.

2. EXPERIMENTAL
Experiments were conducted on *Glycine max* and *Phaseolus vulgaris.* The seeds were obtained from local Market. Healthy seeds of both species were selected and sterilized with 0.1% Mercuric chloride solution for 5 minutes followed by rinsing with tap and distilled water. Seeds were sown in 8cm diameter plastic pots containing 300gm of sterilized soil.Half-strength Hoagland's solution was used to irrigate the plants throughout experimental period[15].Two weeks old seedlings were exposed to two different 2 and 3% levels of CO_2in controlled environment chamber for 40 days. Air is drawn into chamber by cylinder which were obtained from "The National Gas Limited Pakistan", enriched with CO_2, and blown through the chamber. All the chambers were maintained at 2 and 3% CO_2 levels at 30°C temperature. Plants were fumigated with elevated CO_2 for 15 minutes per day for 40 days duration, inside chamber light was deliver by fluorescent

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and luminescent lamps, which adjusted at top of the canopy. After CO_2 exposure the pots were returned to natural environmental condition in the net house for growth. The non treated plants serve as control. There were there replicates for each treatment.

2.1 *Plants responses measurements*

Control and treated leaf samples were collected in early hours of the morning and were kept in labeled sample bags. The plants samples were analyzed for following biochemical parameters.

2.2 *Estimation of carbohydrate content*

Carbohydrate content was measured according to the method of Yemm & Willis[16] method using anthrone reagent. 1.0 gm of leaves was homogenized in 10 ml of distilled water and centrifuged at 500 rpm for 5 minutes. The supernatant was used for estimation of total carbohydrate content. The reaction mixture contained 0.5 ml of supernatant and 5 ml of anthrone reagent which was boiled at 100°C for 30 minutes. Absorbance was determined at 620nm. The carbohydrate content expressed as $\mu g.mg^{-1}$ fresh weight.

2.3 *Estimation of peroxidase activity*

The activity of Enzyme peroxidase was assayed according to the method of Alvarez[17]. 0.5g leaf were ground ice chilled 3ml of 0.15M phosphate buffer of pH 5.6 then drop of 3mM Sodium EDTA was added after that the extract was centrifuged at 4000 rpm for 10 minutes at 0°C in a refrigerated centrifuge, the supernatant was used for the estimation of peroxidase activity and soluble protein. Soluble protein was estimated using Folin reagent and Bovine serum albumin using as standard reference (Lowry *et al.*,. 1951)[18]. For determination of peroxidase activity 0.5ml of crude enzyme extract was mixed with 2.5ml of 0.15M phosphate buffer (pH 5.6) and then 0.25ml of 0.1M H_2O_2 and 0.01M pyrogallol was added and after incubation at 28°C absorbance were recorded at 410nm. Activity of peroxidase expressed as $\mu g.\ mg^{-1}\ min^{-1}$ of leaf fresh weight.

2.4 *Extraction and Estimation of protein*

Estimation of Protein was done in plant extracts by the method of Lowry et al[18]. For protein assays, 0.5g leaves were ground in 10ml of distilled water. The homogenates were centrifuged for 10 minutes at 3000rpm and the resulting supernatants were used for determination of protein content assays. To 0.2ml of plant sample, add 3ml alkaline copper sulfate in the presence of tartrate, letthe mixture stand at room temperature for 30minutes. This incubation is then followed by the addition of Folin reagent furthermore read the absorbance at 750nm and Bovine serum albumin is used to make the standard curve. The amount of total protein content was calculated from a standard curve of Bovine serum albumin and expressed as $\mu g.\ mg^{-1}$ of leaf fresh weight.

2.5 *Amino acid Estimation*

Amino acid were extracted and estimated by the method given by Harbon[19], 1.0g of plant material were heat at 60°C for 10 minutes in 10ml of 80% ethanol then material kept over night. Next day it was centrifuged twice at 4000rpm and supernatant was taken in vial and their volume is reduced up to 1ml at 40°C temperature then in reduce extract 1ml of 50% ethanol was added. Separation of amino acids were done by using thin layer chromatography (TLC) technique, on thin layer of silica gel amino acid extract were loaded and run in n-butanol - acetic acid -water (4:1:1) as solvent. After chromatogram dried at room temperature spraying was done with 2% ninhydrine in acetone then spraying the plate and heated for 10 minutes at 105°C when most amino acids give purple or grey-blue color their Rf values were calculated. Standard of amino acids were prepared by dissolving 250µg amino acid in 1 ml absolute ethanol.

3. STATISTICAL ANALYSIS

The data for protein, carbohydrate and peroxidase activity of *Glycine max* and *Phaseolus vulgaris* under different treatments were analyzed using the "COSTAT" statistical program by two-way analysis of variance (ANOVA) to compare the means of different treatment and "SIGMA PLOT" program was used for graphic presentation of the data.

4. RESULTS

The results achieved for the effect of different concentration of CO_2 on total carbohydrate content of *Glycine max* and *Phaseolus vulgaris* leaves are shown in Fig. 1. Significant (**$P<0.01$) increase in total carbohydrate content was observed in treated samples as compared to control samples throughout experimental period. The result for the effect of elevated level of CO_2 treatment on enzymatic activity of peroxidase of *Glycine max* and *Phaseolus vulgaris* are showing in Fig. 2,

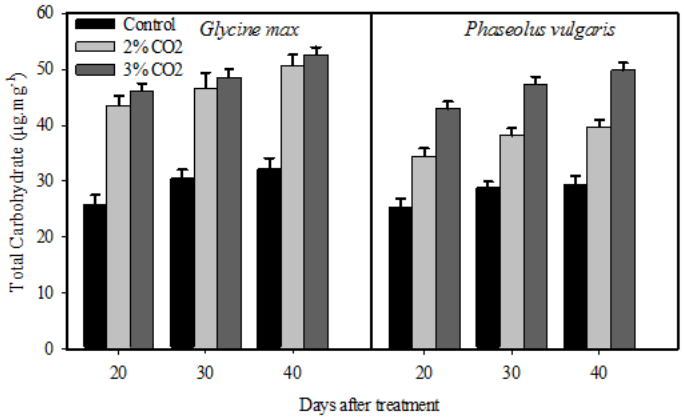

Fig-1: Total carbohydrate content in *Glycine max* and *Phaserlus vulgaris* leaves grown under different Carbon dioxide concentration.

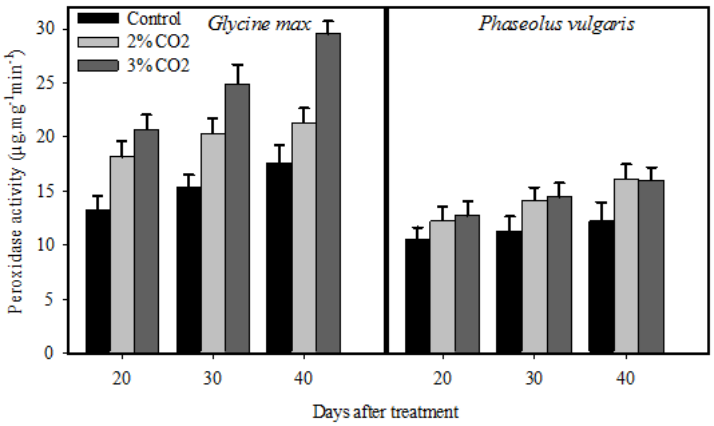

Fig-2: Peroxidase activity in *Glycine max* and *Phaseolus vulgaris* leaves grown under different Carbon dioxide concentration

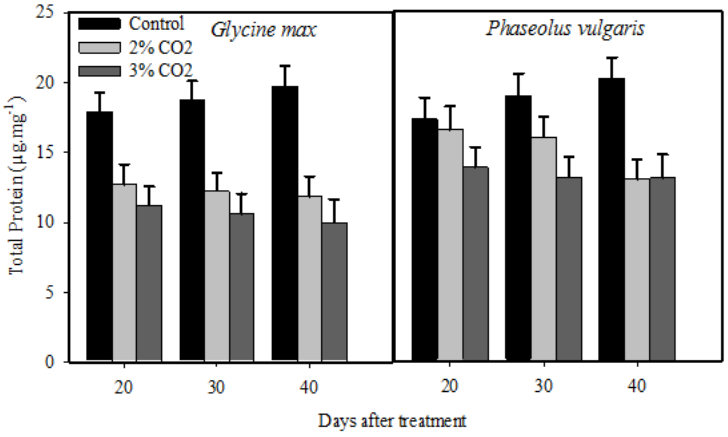

Fig-3: Total protein content in *Glycine max* and *Phaseolus vulgaris* leaves grown under different Carbon dioxide concentration

the result obtained were statistically significant ($P<0.01$). Both CO_2 treatment of *Glycine max* and *Phaseolus vulgaris* demonstrate an increase in activity of peroxidase as compare to control.

The result get for the effect of different concentration of CO_2 on total protein content of *Glycine max* and *Phaseolus vulgaris* are shown in Fig. 3. From Fig. 3 it is clear that Significant decrease in amount of total protein in leave of *Phaseolus vulgaris* ($P<0.05$) and *Glycine max* ($P<0.01$)was observed throughout the experimental period, whereas, increase was observed in control of both species. Individual amino acids content of *Glycine max* were showing in Table 1, at 20th day tyrosine and tryptophan were absent in *Glycine max*. Lycine, proline, valin and phenylalanine were absent only

in control while their traces were present in CO_2 treatment. Traces of threonine and asparagines present in control and 3% CO_2 however they absent in 2% CO_2 treatment. Arginine, alanine, cystine and histidine traces, but low quantity of aspartate, glutamate and glutamine were found in both CO_2 treatments as well as in control of *Glycine max*. In control of *Glycine max* glycine and serine was found in low amount whereas their trace were present in both CO_2 treatment. Moderate amount of methionine, leucine and isoleucine was found in 2 and 3% CO_2 treatment furthermore their traces were found in control of *Glycine max*.

At 30th and 40th day arginine and tyrosine were absent whereas traces of cystine, threonine, glycine, alanine, valine, methionine and phenylalanine and low amount of glutamate and glutamine and aspartate were present in control and both CO_2 treatment of *Glycine max*, rest of the amino acids illustrate the uneven trend.

Individual amino acids content of *Phaseolus vulgaris* were presented in Table 2, at 20th day *Phaseolus vulgaris* illustrate bit lower amount i.e. traces of Cystine, asparagines, alanine, valine, metheonine, leucine and glutamine were present while isoleucine and tyrosine were absent in *Phaseolus vulgaris,* in addition histidine and serine were present in traces in control, but lacking in both CO_2 treatment of *Phaseolus vulgaris*. Traces of lycine, arginine, prolein, phenylalanine, tryptophan and aspartate were found equally in 2% and 3% CO_2 treatments while missing in control of *Phaseolus vulgaris*. In both CO_2 treatment and control of *Phaseolus vulgaris* glutamate was found in low amount whereas threonine and glycine were present in control but lacking in CO_2 treatments.

At 30th day cystine, histidine, aspartate, threonine, asparagines, methoinie, serine and glutamine traces but moderated amount of glycine, glutamate and arginine were present while lycine, tyrosine, proline, tryptophan, alanine, valine, phenylalanine, leucine and isoleucine were missing in control of *Phaseolus vulgaris*. In 2% CO_2 treatment cystine, tyrosine and serine were absent while the traces of all other amino acid were present in *Phaseolus vulgaris.* Cystine, histidine, arginine, methioine, glutamine, phenylalanine, leucine, alanine and valine were present in traces, while low quantity of aspartate, glutamate, proline and asparagines were present in 3% CO_2 treatment of *Phaseolus vulgaris*.

At 40th day amount of various amino acids were found in moderate amount in 2% and 3% CO_2 treatments of *Phaseolus vulgaris* while in control of *Phaseolus vulgaris* traces of various amino acids were observed.

Table-1: Changes in the free amino acids quality in leaves of *Glycine max* under different concentration of CO_2

Amino Acids	**Control**			**2% CO_2**			**3% CO_2**		
	Days after the treatment application								
	20	30	40	20	30	40	20	30	40
Cystine	+	+	+	+	+	+	+	+	+
Histidine	+	+	+	+	+	+	+	-	-
Lycine	-	+	-	+	+	+	+	+	+
Aspartate	++	+	++	++	++	++	++	++	++
Threonine	+	+	+	-	+	+	+	+	+
Glycine	++	+	+	+	+	+	+	+	+
Glutamate	++	++	++	++	++	++	++	++	++
Arginine	+	-	-	+	-	-	+	-	-
Tyrosine	-	-	-	-	-	-	-	-	-
Proline	-	-	-	+	+	++	+	+	++
Asparagine	+	+	+	-	-	-	+	+	+
Tryptophan	-	-	-	-	-	-	-	+	+
Alanine	+	+	+	+	+	+	+	+	+
Valine	-	+	+	+	+	+	+	+	+
Methionine	+	+	+	+++	+	+	+++	+	+
Phenylalanine	-	+	+	+	++	++	+	+	+
Leucine	+	-	-	+++	+	-	+++	+	+
Serine	++	++	++	+	+	+	+	-	-
Isoleucine	+	-	-	+++	+	+	+++	-	++
Glutamine	++	++	++	++	+	++	++	++	++

+ Traces, ++ low, +++ moderate and ++++ high.

Table-2: Changes in the free amino acids quality in leaves of *Phaseolus vulgaris* under different concentration of CO_2

Amino Acids	Control			2% CO_2			3% CO_2		
	Days after the treatment application								
	20	30	40		20	30	40		20
Cystine	+	+	+	+	-	+++	+	+	-
Histidine	+	+	+	-	+	+++	-	+	+++
Lycine	-	-	+	+	+	++	+	-	-
Aspartate	-	+	+	+	+	+++	+	++	+++
Threonine	+	+	+	+	+	+++	-	-	+++
Glycine	++	++	+	+	+	+	-	-	-
Glutamate	++	++	+	++	+	++	++	++	-
Arginine	-	++	+	+	+	+++	+	+	+++
Tyrosine	-	-	-	-	-	-	-	-	++
Proline	-	-	-	+	+	++	+	++	+
Asparagine	+	+	+	+	+	+	+	++	++
Tryptophan	-	-	+	+	+	+++	+	-	+++
Alanine	+	-	+	+	+	++	+	+	-
Valine	+	-	+	+	+	-	+	+	++
Methionine	+	+	+	+	+	++	+	+	+++
Phenylalanine	-	-	-	+	+	+++	+	+	++
Leucine	+	-	-	+	+	+	+	+	++
Serine	+	+	+	-	-	-	-	-	-
Isoleucine	-	-	-	-	+	++	-	-	-
Glutamine	+	+	-	++	+	+++	+	+	+++

+ Traces, ++ low, +++ moderate and ++++ high

5. DISCUSSION

In present investigation increased in total carbohydrate content was observed. Elevated CO_2 conditions produced an average increase in total carbohydrate contents of 28% for clover and 16% for phalaris[20]. Short-term exposure of elevated CO_2 for plants generally leads to increased rates of leaf-level photosynthesis due to better activity of ribulose-1.5-bisphosphate carboxylase/oxygenase[21]. Many studies reported that during growth under twice-ambient CO_2, leaf soluble carbohydrate content increased on average by 52% and starch content by 160% [21]. In the short term (hours to days), elevated CO_2 increases the rate of photosynthesis in plants. However, over the longer term (days to weeks), growth in elevated CO_3 often decreases photosynthetic capacity because of decreased in the content of photosynthetic enzymes[22]. According to an other report an increased in total carbohydrate content of two *Vigna* species was observed, which exposed to enriched CO_2 air for short duration[23].

Increase in peroxidase activity of *Glycine max* and *Phaseolus vulgaris* revealed in present investigation. Short term exposure of enriched CO_2 is reported to enhance the activity of peroxidase[24]. Peroxidase is involved in numerous physiological mechanisms and it is commonly reported that frequently oxidizing agents are responsible for the stimulation of peroxidase activity in plants[25]. Peroxidase is believed to assist the production and breakdown of hydrogen peroxide and other reactive oxygen species[26-28]. Elevated level of CO_2 and ozone, alone and in combination with one another, found to increase the activity of peroxidase, which is the first line of enzymatic defense against oxidative stress, Thus, atmospheric CO_2 enrichment increased the activities of peroxidase enzymes that function to keep cells from experiencing oxidative damage, particularly to their membranes[29].

In the present investigation, decrease in the total protein content is evident with fumigation of the elevated levels of CO_2,however increased in various amino acid content was observed on one hand, on the other lower glycine and serine was observed. Elevated levels of CO_2 caused significant reduction in protein content in wheat[30]. Barbehenn suggested that plants protein concentration decreased much more in the C_3 species (22%) than in the C_4 species (7%) when the plants were grown in CO_2 enriched air[31]. Likewise, Agrawal and Deepak[32] determined that CO_2 enrichment results in diminish leaf protein levels by 3-4% in wheat. Zeigler suggested that such decrease could be attributed to break down of existing protein and reduced in synthesis[33]. Rise in the atmospheric CO2 content typically lead to greater decreases in the concentrations of nitrogen and, therefore, protein in the foliage of C_3 as compared to C_4 grasses[34]. Another investigation supported that the CO_2-induced decrease in leaf protein concentration but it did not reduces the growth rate of plants[35]. Rogers et al. found that total amino acid and Nitrogen content increased markedly at elevated level of CO_2 [36]. Although Amino acids involved in photorespiration (glycine, serine) make a large contribution to the total amino acid pool; as a consequence of the lower rates of photorespiration these amino acids typically decrease at elevated level of CO_2[37].

Ferrario-Mery et al suggested that, reducing photorespiration at elevated CO_2 level may result in large decreases in leaf concentration of glycine and serine and ammonium[38]. Stitt & Krapp recommended that decreased in pool sizes of glycine and serine furthermore associated with lower protein content in leaves at elevated CO_2 (37). Reduced photorespiration decreases the rate of nitrate photo reduction and this may contribute to lower protein content in leaves that grow at enriched CO_2 circumstance[39].

6. CONCLUSION

Exposure of elevated level of CO_2 led to increased carbohydrate content of both legume species. The decrease in protein content and free amino acids (glycine and serine) of *Glycine max* and *Phaseolus vulgaris* was observed. While activity of Peroxidase enzyme, which participate in defense mechanisms of plants under oxidative stress, increases at enriched CO_2 condition.

7. REFERENCE

1. Taiz, L., Zeiger, E., Plant physiology (3rd edition). Sinauers Publ. Co. Inc. Calif, **(2002)**.
2. Salisbury, F. B., Ross, C. B., Plant Physiology (5th Edition), Wadsworth Publishing Co. Belmont CA, **(1992)**.
3. Yong, J. W. H., Lim, E. Y. C., Hew, C. S., Can we use elevated CO_2 to increase productivity in the orchid industry?, Malayan Orchid Review (**2002)** 36: 75-81.
4. Bender, J., Herstein, U., Black, C. R., Growth and yield responses of spring wheat to increasing carbon dioxide, ozone and physiological stresses: a statistical analysis of 'ESPACE-wheat' results. European Journal of Agronomy. **(1999)** 10: 185-195, http://dx.doi.org/10.1016/S1161-0301(99)00009-X.
5. Bowes, G., Growth at elevated CO_2: photosynthetic responses mediated through Rubisco. Plant Cell & Environ **(1991)** 14: 795–806, http://dx.doi.org/10.1111/j.1365-3040.1991.tb01443.x.
6. Wall, G. W., Brooks, T. J., Adam, N. R., Cousins, A., Kimball, B. A., Pinter, P. J. J. R., LaMorter, R. C., Elevated atmospheric CO2 improved sorghum plant water status by ameliorating the adverse effects of drought. New Phyiol **(2001)** 152: 231–248, http://dx.doi.org/10.1046/j.0028-646X.2001.00260.x.
7. Leakey, A. D. B., Bernacchi, C. J., Dohleman, F. G., Ort, D. R., Long, S. P., Will photosynthesis of maize (Zea mays) in the US Corn Belt increase in future CO_2 rich atmospheres? An analysis of diurnal courses of CO_2 uptake under free-air concentration enrichment (FACE). Glob Change Biol **(2004)** 10: 951–962, http://dx.doi.org/10.1111/j.1529-8817.2003.00767.x.
8. Leakey, A. D. B., Uribelarrea, M., Ainsworth, E. A., Naidu, S. L., Rogers, A., Ort, D. R., Long, S. P., Photosynthesis, productivity and yield of maize are not affected by open-air elevation of CO_2 concentration in the absence of drought. Plant Physiol **(2006)** 140: 779–790, http://dx.doi.org/10.1104/pp.105.073957.
9. Long, S. P., Ainsworth, E. A., Leakey, A. D. B., Nosberger, J., Ort, D. R., Food for thought: lower-than-expected crop yield stimulation with rising CO_2 concentration. Science **(2006)** 312: 1918–1921, http://dx.doi.org/10.1126/science.1114722.
10. Breuer, G., Air in Danger: Ecological Perspectives of the Atmosphere. New York, Cambridge University Press, **(1980)**.
11. Orcutt, D. M., Nilsen, E. T., Hale, M. G., The physiology of plants under stress; soil and biotic factors. The Quarterly Review of Biology, University of Chicago press. New York, **(2000)**.
12. Hsiao, T. C., Jackson, R. B., Interactive effects of water stress and elevated CO2 on growth, photosynthesis, and water use efficiency. Carbon dioxide and environmental stress. Academic Press, London, **(1999)**.
13. Hegerl, G. C., Zwiers, F. W., Braconnot, P., Gillett, N. P., Luo, Y., Marengo, Orsini, J. A., Nicholls, N., Penner, J. E., Stott, P. A., Understanding and Attributing Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, **(2007)**.
14. Minorsky, P. V., The hot and classic: Global warming effects on plants. Plant Physiol **(2002)** 129: 1421-1422, http://dx.doi.org/10.1104/pp.900042.
15. Hoagland, D. R., Arnon, D.I., The water-culture method for growing plants without soil. California Agricultural Experimental Station Circular **(1950)** 347: 1-32.
16. Yemm, E. W., Willis, A. J., The Estimation of Carbohydrate in the Plant Extract by Anthrone Reagent. Journal of Biochemistry **(1954)** 57: 508-514.
17. Alvarez, M. R., Temporal saptial changes in peroxidase activity during fruit development in EncycliaTampensis (Orchidaceae). Amer J Bot **(1968)** 55(5): 619-625, http://dx.doi.org/10.2307/2440617.
18. Lowry, O. H., Rosbrough, N. J., Farr, A. L., Randall, R. J., Protein measurement with the folin phenol reagent. J BiolChem **(1951)** 193: 265.

19. Harbone, J. B., Phytochemical methods, 2nd ed. Chapman and Hall, London, **(1984)**, http://dx.doi.org/10.1007/978-94-009-5570-7.
20. Lilley, J. M., Bolger, T. P., Peoples, M. B., Gifford, R. M., Nutritive value and the nitrogen dynamics of Trifoliumsubterraneumand Phalarisaquaticaunder warmer, high CO2 conditions. New Phytol **(2001)** 50: 385–95, http://dx.doi.org/10.1046/j.1469-8137.2001.00101.x.
21. Moore, B. D., Cheng, S. H., Sims, D., Seemann, J. R., The biochemical and molecular basis for photosynthetic acclimation to elevated atmospheric CO2. Plant Cell Environ **(1999)** 22 (6): 567- 582, http://dx.doi.org/10.1046/j.1365-3040.1999.00432.x.
22. Griffin, K. L., Seemann, J. R., Plants CO_2 and pholosynthesis in the 21st century. Chemistry and Biology **(1996)** 3: 245-254, http://dx.doi.org/10.1016/S1074-5521(96)90104-0.
23. Hamid, N., Jawaid, F., Amin, D., Effect of short-term exposure of two different carbon dioxide (CO_2) concentrations on growth and some biochemical parameters of edible beans (Vignaradiata and Vignaunguiculata) Pak. J. Bot **(2009)** 41(4): 1831-1836.
24. Lambreva, M., Christov, K., Tsonev, T., Short-term effect of elevated carbon dioxide CO_2 concentration and high irradiance on the antioxidant enzyme in bean plants. Biolplantarum **(2006)** 50(4): 617-623.
25. Nesser, L. E. A. A., Effect of ozone and stimulated acid rain on growth, nitrogen fixation and peroxidase activity in Faba Bean (Viciafaba) plant. Asian journal of plant sciences **(2002)** 1(4): 456-461, http://dx.doi.org/10.3923/ajps.2002.456.461.
26. Legendre, L., Rueter, S., Heinstein, P. F., Low, S. P. Characterization of the oligogalacturonide-induced oxidative burst in cultured soybean (Glycine max) cells. Plant Physiol **(1993)** 102: 233–240, http://dx.doi.org/10.1104/pp.102.1.233.
27. Klotz, K.L., Lagrimini, L. M., Phytohormone control of the tobacco anionic peroxidase promoter. Plant MolBiol **(1996)** 31: 565–573, http://dx.doi.org/10.1007/BF00042229.
28. Cipollini, D. F., JR. The induction of soluble peroxidase activity in bean leaves by wind-induced mechanical perturbation. Am J Bot **(1998)** 85(11): 1586–1591, http://dx.doi.org/10.2307/2446485.
29. Niewiadomska, E., Gaucher-Veilleux, C., Chevrier, N., Mauffette, Y., Dizengremel, P., Elevated CO2 does not provide protection against ozone considering the activity of several antioxidant enzymes in the leaves of sugar maple. J Plant Physiol **(1999)** 155: 70-77, http://dx.doi.org/10.1016/S0176-1617(99)80142-4.
30. Wieser, H., Manderscheid, R., Erbs, M., Weigel, H. J., Effects of elevated atmospheric CO_2 concentrations on the quantitative protein composition of wheat grain. J AgricFoodchem **(2008)** 56(15): 6531-6535, http://dx.doi.org/10.1021/jf8008603.
31. Barbehenn, R. V., Karowe, D. N., Chen, Z., Performance of a generalist grasshopper on a C_3 and a C_4 grass: compensation for the effects of elevated CO_2 on plant nutritional quality. Oecologia **(2004)** 140: 96-103, http://dx.doi.org/10.1007/s00442-004-1555-x.
32. Agrawal, M., Deepak, S. S., Physiological and biochemical responses of two cultivars of wheat to elevated levels of CO2 and SO2, singly and in combination. Environ Pollut **(2003)** 121: 189-197, http://dx.doi.org/10.1016/S0269-7491(02)00222-1.
33. Zeigler, I., The effect of SO_2 pollution on plant metabolism. Residue Rev **(1975)** 56:79 -105.
34. Wand, S. J. E., Midgley, G. F., Jones, M. H., Curtis, P. S. Responses of wild C_4 and C_3 grass (Poaceae) species to elevated atmospheric CO_2 concentration: a meta-analytic test of current theories and perceptions. Global Change Biology **(1999)** 5: 723-741, http://dx.doi.org/10.1046/j.1365-2486.1999.00265.x.
35. Barbehenn, R. V., Karowe, D. N., Spickard, A., Effects of elevated atmospheric CO_2 on the nutritional ecology of C_3 and C_4 grass-feeding caterpillars. Oecologia **(2004)** 140: 86-95, http://dx.doi.org/10.1007/s00442-004-1572-9.
36. Rogers, A., Gibon, Y., Stitt, M., Morgan, P. B., Bernacchi, C. J., Ort, D. R., Long, S. P., Increased C availability at elevated carbon dioxide concentration improves N assimilation in a legume. *Plant Cell Environ* **(2006)**29: 1651-1658, http://dx.doi.org/10.1111/j.1365-3040.2006.01549.x.
37. Stitt, M., Krapp, A., The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. Plant Cell Environ **(1999)** 22: 583–621, http://dx.doi.org/10.1046/j.1365-3040.1999.00386.x.
38. 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 and transformed Nicotianaplumbaginifolia during CO2 enrichment of plants grown in pots and in hydroponic culture. Planta **(1997)** 202: 510-521, http://dx.doi.org/10.1007/s004250050156.
39. Rachmilevitch, S., Cousins, A. B., Bloom, A. J,. Nitrate assimilation in plant shoots depends on photorespiration. ProcNatIAcadSci USA **(2004)** 101: 11506-11510, http://dx.doi.org/10.1073/pnas.0404388101.