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Research Article

CARBON SEQUESTRATION ON GERMINATION OF MAIZE UNDER CONTROLLED CONDITION IN OPEN TOP CHAMBER

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Abstract: An enzymal activity experimental study was done in maize (*Zea mays* L.) of variety $COHM_5$ using arbuscular mycorrhizae (AM) inoculated(M+) and non-inoculated with Glomus intraradices. Roots and shoots sampled at 15, 30 and 45 days after sowing (DAS) were estimated for total chlorophyll content, peroxidase, catalase, phenol and polyphenol oxidase, humic acid, fulvic acid, Biomass C, Biomass N, Pep case and soluble protein. Elevated CO_2 with mycorrhizal inoculation significantly increases total chlorophyll content, antioxidative enzymes (peroxidase, catalase, phenol and polyphenol oxidase), humic acid, fulvic acid, Biomass C, Biomass N. But in case of Pep case and soluble protein activity increases in 370 ppm than 550 and 750 ppm its because of denaturation of enzyme activity was more pronounced as crop duration increases with the inoculation of VAM.

Keywords: Climate change, CO_2 enrichment, C_4 photosynthesis, Antioxidative enzymes

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Introduction

With the advent of global warming there is an increasing trend of carbondioxide concentration was predicted to be raised from 370-550ppm by 2050 and 730-1010 ppm by 2100 [22]. An experimental study was conducted with yield aspect of crops to the carbondioxide enrichment and concluded a significant yield increment [9]. It was predicted that change in weather brings about change in climate which in turn leads to increase in temperature by around 30C by 2050. Increased atmospheric carbondioxide concentrations shows differential effects among different crop species [8, 15].C4 crops considered important in world are maize, sorghum, forage and range grasses *i.e.*, Panicum maximum and noxious weed particularly *Echinochloa* and *Amaranthus* spp [2] contributes approximately 4% of world's plant species and it accounts to about 18-21% global productivity because of its capability of its higher productivity [6, 14]. It was concluded that elevated CO_2 brings about enhanced above ground crop growth which indicate potential for improvements in soil carbon storage, water infiltration and soil water retention and reduced erosion [17]. For net assimilation rate, the increases were smaller, but fell with time in a similar way. The C_4 crops responded very much less than C_3 crops. The responses of biomass accumulation and yield were similar to that for carbon fixation rate. Yield increased on average 41% for a doubling of CO_2 concentration. In particular to the harvest index there was small change and decreases with increasing amount of CO_2 concentration. Moreover, when dealing with the aspects like conductance and transpiration is inversely proportional with CO_2 concentration. Heat stress induces significant changes in normal physio-logical processes such as photosynthesis dark respiration, membrane stability and mitochondrial respiration [16]. High temperature injury can result in considerable pre-harvest and post-harvest crop losses. One mechanism of injury involves the generation and reactions of reactive oxygen species (ROS) [13]. In order to limit oxidative damage under stress condition plants have developed a series of detoxification systems that break down the highly toxic ROS [11]. Plants protect cell and subcellar systems from the cytotoxic effects of the active oxygen radicals using antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, glutathione reductase, catalase and metabolites like glutathione, ascorbic acid, a-tocopherol and carotenoids [20].

Materials and Methods

Pot culture experiment was conducted in open top chamber (Fig 1) at different levels of carbondioxide such as 370 ppm, 550ppm and 750 ppm respectively at the Department of soil science and agricultural chemistry, Tamil Nadu Agricultural University, Coimbatore. Soil samples were collected, processed and autoclaved in order to eliminate the indigenous mycorrhizal fungal population. The soil had sandy loamy texture with slightly alkaline (pH 7.8) and low in available N and medium in available P and K, respectively. There were 6 treatment combinations replicated six times in a completely randomized block design (CRBD). Thirty six pots containing 2 kg of soil was taken for the experiment. Twelve pots are kept in 370ppm out of twelve pots six pots are inoculated with VAM and without VAM. Twelve pots are kept in 550ppm out of twelve pots six pots are inoculated with VAM and without VAM. Twelve pots are kept in 750ppm out of twelve pots six pots are inoculated with VAM and without VAM. The arbuscular mycorrhizal fungal inoculum carrying Glomus intraradices (2g) was applied at the base of the seed hole just prior to sowing. Mycorrhizal inoculum mixed with vermiculite was grown in maize plants which consist of infected root bits, spores and then this strain was mixed with sterile vermiculite. Maize hybrid seeds (COMH-5) were sown on the inoculum layer of soil. Germination percentage was nearly 95% on the seventh day of sowing. Half the dose of N (75 kg ha^{-1}) and full dose of P (75 kg ha^{-1}) and K (75 kg ha^{-1}) were applied in the form of urea, single superphosphate and muriate of potash, respectively, as basal at the time of sowing. In this experiment root colonization, chlorophyll, plant, root and soil biochemical changes were measured. The data collected were statistically analyzed using ANOVA. The plant biochemical such as soluble protein, pepcase, Shoot biomass, root biomass and peroxidase, root biochemical such as phenol, polyphenol oxidase and biochemical parameters such as Humic and fulvic acid , Biomass C and Biomass N and Glomalin were assessed for 15th, 30th and 45th days .

Table-1 Impact of elevated CO_2 on chlorophyll content in leaves of maize hybrid (COHM5) (mg/g of tissue)

Treatments VAM inoculation & CO2 levels (ppm)	Chla (mg/g of tissue) Days after sowing			Chlb(mg/g of tissue) Days after sowing			Totalchl (mg/g of tissue) Days after sowing		
	15	30	45	15	30	45	15	30	45
M- (Without VAM inoculation)									
370ppm	0.93	1.86	2.8	1.73	3.45	5.18	2.66	5.32	9.66
550ppm	0.69	1.39	2.08	1.28	2.77	3.12	1.97	4.16	8.13
750ppm	0.64	1.31	1.93	1.18	2.36	3.54	1.82	3.67	9.21
M+ (With VAM inoculation)									
370ppm	1.1	2.19	3.29	2.12	4.25	6.37	3.22	6.44	7.98
550ppm	1.04	1.92	2.88	2.03	3.5	5.26	3.07	5.42	5.2
750ppm	0.96	2.07	3.11	1.75	4.06	6.1	2.71	6.14	5.47
SED									
M	0.017	0.034	0.052	0.067	0.134	0.202	0.106	0.162	0.23
C	0.021	0.042	0.064	0.082	0.165	0.247	0.13	0.197	0.282
M*C	0.03	0.06	0.09	0.116	0.233	0.35	0.184	0.279	0.4
CD (0.05)									
M	0.035	0.071	0.107	0.138	0.277	0.416	0.218	0.332	0.475
C	0.044	0.088	0.132	0.169	0.34	0.51	0.267	0.407	0.582
M*C	0.062	0.124	0.186	0.24	0.481	0.721	0.378	0.576	0.823

Table-2 Impact of elevated CO_2 on biomass of root and shoot of maize hybrid (CoHM5)

Treatments VAM inoculation & CO_2 levels (ppm)	Shootbiomass (gm) Days after sowing			Rootbiomass(gm) Days after sowing		
M-(Without VAM inoculation)						
	15	30	45	15	30	45
370ppm	7.85	15.70	23.55	0.17	0.33	0.50
550ppm	12.68	25.37	38.05	0.27	0.55	0.82
750ppm	16.28	32.57	48.85	0.31	0.62	0.94
M+ (With VAM inoculation)						
370ppm	10.32	20.63	30.95	0.23	0.47	0.70
550ppm	15.32	30.63	45.95	0.34	0.67	1.02
750ppm	20.28	40.57	60.85	0.37	0.73	1.10
SED						
M	0.093	0.187	0.280	0.005	0.012	0.017
C	0.114	0.229	0.343	0.007	0.015	0.021
M*C	0.162	0.324	0.486	0.010	0.021	0.030
CD(0.05)						
M	0.192	0.385	0.578	0.012	0.025	0.036
C	0.236	0.472	0.708	0.014	0.031	0.044
M*C	0.333	0.667	1.001	0.021	0.044	0.063

Table-3 Impact of elevated CO_2 on root biochemical changes on maize hybrid (CoHM5)

Treatments VAM inoculation & CO_2 levels (ppm)	Peroxidase(Change in OD/min/g)			Catalase(μgH_2O_2remain/g/min)			Phenol(%freshwt)			Polyphenoloxidase (Change in OD/min/g)		
M-(Without VAM inoculation)												
	15 DAS	30DAS	45DAS	15DAS	30DAS	45DAS	15DAS	30DAS	45DAS	15DAS	30DAS	45DAS
370ppm	0.25	0.5	0.75	0.73	1.46	2.2	0.21	0.42	0.64	0.54	0.42	0.64
550ppm	0.16	0.32	0.49	0.53	1.06	1.58	0.13	0.39	0.39	0.47	0.26	0.39
750ppm	0.11	0.22	0.34	0.43	0.86	1.3	0.11	0.22	0.33	0.37	0.22	0.33
M+(With VAM inoculation)												
370ppm	0.32	0.64	0.96	0.83	1.65	2.48	0.25	0.5	0.76	0.72	0.5	0.76
550ppm	0.24	0.49	0.73	0.63	1.25	1.88	0.17	0.35	0.52	0.66	0.35	0.58
750ppm	0.16	0.31	0.43	0.54	1.08	1.62	0.13	0.26	0.4	0.58	0.26	0.4
CD (0.05)												
M	0.01	0.021	0.032	0.009	0.018	0.029	0.007	0.014	0.021	0.007	0.014	0.015
C	0.013	0.026	0.039	0.011	0.022	0.036	0.008	0.017	0.026	0.008	0.017	0.018
M*C	0.018	0.037	0.055	0.015	0.031	0.05	0.012	0.024	0.036	0.012	0.024	0.026

Chlorophyll content in the plant samples was measured by following procedure of [3]. Polyphenol oxidase was measured by [4]. Humic acid and fulvic acid was weighed and reported as percentage in soil [23]. The biomass carbon and Biomass Nitrogen was determined by the fumigation–incubation technique [10]. Glomalin extractions from soil were carried out as described by [25].

Plant Biochemical changes

Shoot and Root Biomass

Shoots and Roots were collected from different levels of carbondioxide concentrations such as 370ppm, 550ppm and 750ppm and their respective weights are taken.

Pepcase

Plant sample of 0.5 gram was macerated in Tris buffer with mercapto ethanol and centrifuged. The supernatant of 0.2 ml was used for blank and sample. The sample was read for every 60 seconds upto 300 seconds.

Soluble protein

Soluble proteins in plant samples were determined by using bovine serum albumin (BSA) as a standard.0.25g of leaf sample was taken and macerated with 10ml of phosphate buffer. Then centrifuged at 3000 rpm for 10mts and the supernatant solution was collected. 1ml of supernatant solution was pipette out into a test tube and 5ml of alkaline copper tartarate reagent was added.

Table-4 Impact of elevated CO_2 on soil humic and fulvic acid changes on maize hybrid (CoHM5)

Treatments VAM inoculation & CO_2 levels (ppm)	Humic acid (%OM)			Fulvic acid (%OM)		
M-(Without VAM inoculation)						
	15DAS	15DAS	30DAS	45DAS	30DAS	45DAS
370ppm	0.07	0.03	0.06	0.09	0.14	0.22
550ppm	0.11	0.05	0.09	0.14	0.21	0.32
750ppm	0.13	0.08	0.16	0.25	0.26	0.40
M+(With VAM inoculation)						
370ppm	0.11	0.05	0.10	0.15	0.22	0.33
550ppm	0.14	0.08	0.15	0.23	0.28	0.43
750ppm	0.16	0.12	0.23	0.35	0.2	0.48
CD(0.05)						
M	0.005	0.006	0.012	0.018	0.010	0.015
C	0.006	0.007	0.014	0.022	0.012	0.018
M*C	0.008	0.010	0.020	0.031	0.017	0.026

Table-5 Impact of elevated CO_2 on changes in total soluble protein and PEP Case activity of maize hybrid (CoHM5)

Treatments VAM inoculation & CO_2 levels (ppm)	PEPcase (µmolm^{-2}s^{-1})			Soluble protein (µg/g)		
M-(Without VAM inoculation)						
	15 DAS	30DAS	45DAS	15DAS	30DAS	45DAS
370ppm	61.50	123	184.50	33.66	67.32	100.98
550ppm	59.40	118.80	178.20	24.80	49.60	49.60
750ppm	56.52	113.03	169.55	20.37	40.75	61.12
M+(With VAM inoculation)						
370ppm	63.55	127.10	190.65	37.43	74.85	112.28
550ppm	60.38	120.77	181.15	32.40	65.09	65.09
750ppm	57.48	114.97	172.45	26.43	52.85	71.28
CD(0.05)						
M	0.086	0.173	0.256	0.536	1.073	1.435
C	0.106	0.212	0.313	0.657	1.314	1.758
M*C	0.149	0.299	0.443	0.929	1.859	2.486

Table-6 Impact of elevated CO_2 on soil biochemical changes on maize hybrid (CoHM5)

Treatments VAM inoculation & CO_2 levels (ppm)	Biomass C (mg/kg)			Biomass N (mg/kg)			Glomalin (mg/g)		
M- (Without VAM inoculation)	15DAS	30DAS	45DAS	15DAS	30DAS	45DAS	15DAS	30DAS	45DAS
370ppm	62.55	125.1	187.65	7.47	14.93	22.4	0.05	0.04	0.03
550ppm	65.58	131.17	196.75	8.52	17.03	25.55	0.07	0.05	0.04
750ppm	68.53	137.07	136.9	10.67	21	32	0.09	0.06	0.05
M+(With VAM inoculation)									
370ppm	65.53	131.07	196.6	10.47	20.93	31.4	0.05	0.04	0.03
550ppm	67.58	135.2	202.75	11,67	23.33	35	0.06	0.05	0.04
750ppm	71.35	142.7	212.7	12.7	25.4	38.1	0.07	0.06	0.05
CD (0.05)									
M	0.104	0.207	0.301	0.087	0.173	0.259	0.001	0.002	0.005
C	0.127	0.254	0.369	0.106	0.212	0.318	0.002	0.004	0.006
M*C	0.18	0.359	0.522	0.148	0.299	0.449	0.003	0.006	0.008

The solution was kept as such for 30 minutes for biuret reaction to take place. Then 0.5ml of Folin ciacalteau reagent was added and the intensity of blue colour was measured in a spectrophotometer at 660nm.

Peroxidase

Leaf sample 0.5 g of was weighed and macerated with 10ml of phosphate buffer. The contents were centrifuged at 5000 rpm for 15 minutes. One ml of supernatant was taken in attest tube and 3ml of pyrogallol was added. The content was transferred to cuvette and it was read as blank in a spectrophotometer. The 0.5 ml of hydrogen peroxide was added as substrate the change in the OD value was recorded at 430 nm for 2 minutes with every 30 seconds interval. The difference in the OD was calculated and the average of the differences was worked out.

Root Biochemical changes / Catalase

0.5 g of root sample was weighed and macerated with 10 ml of phosphate buffer. The contents were centrifuged at 3000rpm for 10 minutes. One ml of supernatant was taken in five different beakers. To this 5 ml of 1.5% sodium perborate and 1.5ml of phosphate buffer was added. Sulphuric acid of 2N concentration as 10 ml volume added to the enzyme extract with different time intervals of 1,2,3 and 4 minutes in all four beakers. But in the last beaker sulphuric acid of 10 ml should be added prior to the enzyme extract and it was treated as for blank value. Finally, the contents are titrated with 0.05N potassium. Pink colour was developed as endpoint which persists for 30 seconds. The volume of Potassium permanganate consumed was noted. One ml of Potassium permanganate is equal to 0.85 µg of hydrogen peroxide. The activity of the enzyme was expressed as µg of hydrogen peroxide /g/minute.

Total Phenol: Fresh root sample of 0.5 g was taken and macerated with 80% ethanol. It was centrifuged at 10000 rpm for 20 minutes and the supernatant was evaporated. The residue was dissolved in 5 ml of distilled water. One ml of the suspension was made upto 3 ml with distilled water and 0.5 ml of folins reagent was added. After 3 minutes 2 ml of 20% of sodium carbonate was added and mixed thoroughly and kept it in boiling water for 1 minute. After bringing the solution to cooling temperature the absorbance was read at 650nm.

Result and Discussion

The data [Table-1] showed that all the chlorophyll parameters increases with increase in duration of days in 370 ppm of CO_2 levels (1.10, 2.19 and 3.29 mg/g respectively on 15th, 30th and 45th days) with VAM inoculation than without VAM inoculation.

As when CO_2 levels increases to 550 ppm and 750 ppm the chlorophyll parameters decrease when compared to control (370 ppm). Finally, the data showed that the plants which are grown with VAM inoculation produced more of chlorophyll than without VAM inoculation [5]. It was observed from data [Table-2] and Fig 2a, b, c and d that when the concentration of the CO_2 levels increases from 370 ppm (30.95 gm), 550 ppm (45.95 gm) and 750 ppm (60.85 gm) the shoot biomass increases with VAM inoculation than without VAM inoculation and it was increase in the number of leaves per plant [1] and similar pattern of increase was also found in root biomass [12]. It was inferred from [Table-3] the magnitude of increase of peroxidase activity was more in 370 ppm in 15th day (0.96 Change in OD/min/g) with VAM inoculation than without VAM inoculation. Similar trend of increase was also found in catalase, phenol and polyphenoloxidase. However, the magnitude of increase of catalase was more in 370 ppm (2.48 µg H_2 O_2 remain/g/min), phenol (0.76 percent fresh weight) and polyphenoloxidase (0.76 Change in OD/min/g) than 550 ppm and 750 ppm. It may be due to denaturation of enzyme activity as duration of days increases [24]. The data in [Table-4] showed that humic acid and fulvic acid increases with increase in concentration of CO_2 levels when VAM is inoculated. The magnitude of increase of humic acid was more in 750 ppm (0.48 percent OM) than 550 ppm (0.43 percent OM) and 370 ppm (0.33 percent OM). Similar pattern of increase was found in fulvic acid. In [Table-5] pepcase decreases with the increase in CO_2 levels and it was found to have highest value in 370 ppm (63.55,127.10 and 190.65 µmolm-2s-1 respectively of 15th, 30th and 45th days) than in 550 ppm and 750 ppm [7]. Similarly, soluble protein also decreases with the increase in CO_2 levels. The highest soluble protein content was more in 370 ppm (37.43, 74.85 and 112.28 µg/g respectively of 15th, 30th and 45th days) than in 550 ppm and 750 ppm [18]. From [Table-6] Biomass C and Biomass N increases with increase in concentration of CO_2 levels when inoculated with VAM. The magnitude of increase of biomass C was more in 750 ppm (212.70 mg/kg) than 550 ppm (202.75 mg/kg) and 370 ppm (196.60 mg/kg) [21]. Same trend of magnitudinal increase was seen in biomass N with respect to the gradient levels of CO_2 [12]. Glomalin increases with increase in concentration of CO_2 levels when VAM is inoculated. Glomalin, an iron containing glycoprotein produced by mycorrhizal fungi as a component of hyphal and spore wall [19] containing 30-40 percent C considered as a major sequester of C and potentially important for the biological activities of soil. The amount of C in glomalin represented 45 percent of total C and comprises as much as 2 percent of soil by weight which might have contributed to the increased soil C under inoculated soil. [19] reported that glomalin concentration was consistently and highly positively correlated with soil C.

Conclusion

The growth response of C_4 plants to elevated carbondioxide is particularly interesting. The near saturation of C_4 as opposed to C_3, photosynthesis at current ambient carbondioxide offer an excellent opportunity to the growth response of plants to CO_2 enrichment. We conclude from available data that CO_2 enrichment can increase growth of C_4 plants. However, this type of short term experiments can be extrapolated to field conditions inorder to know the actual effect on its degree. However, in future, long term studies in less-disturbed soils are needed to determine whether CO_2 enhancement significantly have limitation over plant growth in elevated CO_2 environment.

Application of research: The paper projects the effects on different levels of Carbondioxide in maize crop in aspects like plant biochemical changes, Chlorophyll content, Humic acid, Fulvic acid, Biomass C and Biomass N, Glomalin

Research Category: Germination of Maize

Abbreviations: VAM-Vesicular arbuscular mycorrhizae, CRBD-completely randomized block design, ROS-reactive oxygen species, CO_2 -Carbondioxide

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