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CROP PRODUCTION

Interaction between CO_2 Elevation and Nitrogen Metabolism in Two Varieties of Guar (*Cyamopsis tetragonoloba*) Plants[1]

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Abstract—The aim of the experimental study is to analyse the consequence of carbon dioxide elevation on activity of nitrate reductase (NR; EC 1.6.6.1), nitrite reductase (NiR; EC 1.7.7.1), glutamate synthetase (GOGAT; EC 1.4.1.13) in leguminous *Cyamopsis tetragonoloba* leaves. Plants were exposed to different atmospheric carbon dioxide concentrations 300 ppm (ambient) and 490 ppm (an elevated) conditions. A decrease in activity of Nitrate Reductase (NR), Nitrite Reductase (NiR), Glutamate Synthetase (GOGAT) was found in elevated condition when compared to ambient condition. Plant nitrogen on dry weight basis was found to decrease under elevation with no significant change in soil nitrogen. Soil pH was found to change significantly under elevation, thus showing a decline in pH and promoting soil acidiosis. Total plant fresh weight (FW) and total plant dry weight (DW), Leaf area/cm^2 were found increased in elevated condition. Thus, in *Cyamopsis tetragonoloba* plant it can be concluded that under carbon dioxide elevation Nitrate reductase, Nitrite reductase and Glutamate synthetase activity is suppressed due to a reduced amount of nitrate translocation and NADH availability for reduction in plant which is correlated with reduction in plant total nitrogen content where as plant growth and biomass is enhanced due to higher carbon fixation.

Keywords: NR, NiR, GOGAT, Guar, FW, DW

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1. INTRODUCTION

The level of carbon dioxide (CO_2) in the atmosphere is tremendously increasing from the last century [1, 2] and its present level is 390 ppm in 2014. It is expected to reach value of 550–600 ppm by 2050. Future projection of increased carbon dioxide emission is due to various anthropogenic human activities, rapid growth and industrialisation. Carbon dioxide is a green house gas as it is the main cause of global warming and climate change [3]. The world population is estimated to reach 8.3 billion by the year 2030, therefore, the demand for food grains is expected to increase by 50% globally (FAO, 2006; World Bank, 2007).To feed such a population globally considerable stress will be imposed on increasing crop productivity due to combination of factors, like limited agricultural land, resource constraint of water and nutrients and rapid global environmental change. To ensure food security globally in the past and present times various assumptions were made and numerous experiments were designed to see the possible effects of increased carbon dioxide on the plant growth, photosynthetic rate and nitrate uptake and nitrogen metabolism. Crop plants are classified as C_3/C_4 on the basis of initial photosynthetic product C_3 plants constitute 95% of plants on earth and remaining 1% are C_4 plants. Since carbon dioxide is the main restrictive aspect of highest photosynthesis in plants [4], its enhanced ambient level leads to increased photosynthesis rate in C_3 plants, that directly affects the proportional increase in growth parameter (41%) and indirectly plant yield [5]. Previous studies carried out under carbon dioxide elevation reported diverse responses in plant growth, plant metabolism and crop yield.

It is now confirmed that elevated carbon dioxide concentrations cause an increase in crop yield and productivity which obviously benefits agriculture on one hand, but results in change in composition of plant tissue nitrogen and carbon content [9] on the other. Carbon (C) and nitrogen (N) are the key struc-

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tural elements for plant growth and constitute 45 and 5% of plant dry matter, respectively [6]. Previous studies reported drastic changes in nitrogen concentration in plants grown under elevated condition which indicate physiological and morphological changes occurring in plant for eg. Limited nitrogen availability alters C:N ratio and it leads to nitrogen limitation as leaves acquire faster carbohydrates than nitrogen, that leads to decrease in nitrogen content in leaves [7]. Early leaf senescence in sunflower leaves is noticed [8] which can be reversed by adequate nitrogen supply [9] that alter gene expression. Earlier studies reported that under enhanced carbon dioxide concentration overall content of plant nitrogen decreases [10]. This decline in the nitrogen in the plant is the indication that the restricted rate of nitrate translocation, its assimilation and photosynthesis occurs and differs greatly. It is found that effect of elevated carbon dioxide concentrations on photosynthetic activity and growth depends on total nitrogen available in plants [11] which indirectly depends on soil nitrogen content. A short term carbon dioxide exposure confirmed an increased in net photosynthesis rate and decreased transpiration [12]. Similarly experiment on Cucumber plant showed increase in carbon dioxide assimilation that increases nitrate reduction by enhancing nitrate assimilation and nitrogen metabolism. Basically elevated carbon dioxide concentration promoted a decrease in "N" uptake in plant and thus stimulating less "N" translocation within the plant. More over during "N" translocation from soil to root and from root to aerial parts "N" uptake and accumulation is noticed more in root than in stem. In case of leguminous plant species elevation concentration promoted higher nitrogen fixation in plant species. Crop plants are categorised into four types based on their preferences for "N" mobilisation. NH_4^+–N, NO_3^-–N, equal effect of both and combinative effect of two N sources being superior to either NH_4^+ or NO_3^- alone. Soil has NO_3^- as primary form of available nitrogen source. Nitrate cannot be mobilised by plants until it is converted to ammonia. Ammonia accumulation is toxic to plant cell as it is able to uncouple respiration at low concentration, so it must be assimilated to less organic form. This all process is under strict control of nitrogen assimilatory pathways. Its reduction is catalysed by two step enzymatic process in which first step occurs in cytoplasm and second step in chloroplast. Nitrate assimilation in plant is firstly achieved in cytoplasm by Nitrate reductase (NR; EC 1.6.6.1), converting NO_3^- to NO_2^-, the key enzyme of nitrogen metabolism. Nitrite reductase (NiR; EC 1.7.7.1) secondly reduces in chloroplast NO_2^- formed to NH_4^+. More over the assimilation of NH_4^+ is incorporated into the organic form is achieved by glutamine/glutamate synthetase pathway [13]. Glutamate synthetase is one of the key enzyme in this process, that catalyzes the transfer of the amide group from glutamine to α-ketoglutarate to yield two molecules of glutamate by amino transferase activity. Glutamate synthetase occurs in two isoforms in higher plants NADH-GOGAT and Ferrodoxin dependent GOGAT(Fd-GOGAT) enzymatic assay [14].

India is the world largest producer of pulses with states Madhya Pradesh (23%) followed by Uttar Pradesh (18%) and Maharashtra (14%) yet we have to meet increasing population demand. Pulses (grain legume) are part of daily intake for humans and animals, rich in protein and fibers. Edible crops productivity of different plant species is affecting on the agriculture land under increasing carbon dioxide concentrations. Free Air Carbon dioxide Enrichment System (FACE) experiments have been conducted in several geographical locations around the world to estimate, under the most realistic agricultural conditions possible, the impact of the carbon dioxide levels projected for the middle of this century on crops species. *Cyamopsis tetragonoloba* a major annual, leguminous and edible kharif crop belongs to the family leguminosea, is cultivated on large scale, 80% of world production in India and Pakistan for its edible pod and as fodder for animals (https://en.wikipedia.org/wiki/Guar), Punjab, Rajasthan, Thar, Gujarat, Kutch region occupies the largest area (82.1%) under guar cultivation in India. In addition to its cultivation in India and the crop is also grown as a cash crop in other parts of the world. It is commonly known as Guar or Cluster bean. It is valuable plant that enhances soil fertility by its symbiosis association with nitrogen fixing bacteria thus Agriculturist in semiarid regions of Rajasthan follow crop rotation and utilises Guar to replenish soil with essential fertilizers and nitrogen fixation before next crop. More over gelling agent containing seeds (GUAR GUM) are today's most important use as it contain 70–80% galactomannan day by day demands are rising for industrial use of Guar Gum in hydraulic fractioning (Oil shale gas).Thus due to such unique features it is interesting to considered it as a model plant for studying the consequences of carbon dioxide elevation effect on plant growth, photosynthetic rate, nitrate uptake and nitrogen metabolism and predict its outcome on production (yield), seed weight, seed quality and biomass production. More over a limited field study on *Cyamopsis* varieties inclined and motivated researcher to assess the impact of carbon dioxide elevation on proteinacious crop compared to other cereals.

1.2. Growth Parameters

To measure plant growth characteristics, *Cyamopsis* varieties were harvested at 60 days after CO_2 exposure. Plant belonging to leguminous family obtains nitrogen from soil in form of NH_4^+, NO_3^- or by nitrogen fixation. Nitrogen fixation is high expenditure

phenomenon as it requires high "C" carbon input [15]. Thus plant mainly rely on soil nitrate or ammonia as it require less "C" carbohydrate resource and is cost effective phenomenon for plants. Previous studies does not provide any convincing evidence in support of increased NR activity under elevated carbon dioxide concentrations although it was found increased slightly in *Vigna radiate* [16] as well as in mustard [17]. Contrary to this a twofold decrease of NR activity was found in maize [18] and wheat [19]. Similarly vermiculture of *Plumbaginifolia* and hydroponic culture of *N. Plumbaginifolia* [10] also reported a decline in NR activity. More over a 30% decline in NiR activity is reported in lettuce and NR [20].

The purpose of our experimental study is to inspect the effect of different carbon dioxide concentrations in leguminous crop *Cyamopsis tetragonoloba* with special emphasis on nitrogen metabolism enzymes (NR, NiR, GOGAT) and plant nitrogen. To accomplish this various morphological and biochemical parameters were analysed for the purpose to study on overall metabolic change occurring in *Cyamopsis tetragonoloba* plant. Our hypothesis is to see the consequences of elevated carbon dioxide concentration is enhancing or restricting N uptake from soil and to predict its N amount in legumes foliage.

2. MATERIALS AND METHODS

2.1. Experimental Setup and Growth Condition

The experimental was conducted in the Free Air Carbon dioxide Enrichment (FACE) setup located in National Botanical Research Institute (N.B.R.I) an urban site (26°55′ N, 80°59′ E) located at main city of Lucknow, India, situated 132 m above sea level, with loamy sandy soil (sand 55%, silt 31%, clay 14%), pH in range of 8.2–8.6 and the electrical conductivity in range of 230–233 $\mu S\ cm^{-1}$. Seeds of *Cyamopsis tetragonoloba* two varieties RGC 1002 and RGC 1066 were taken from the belt of Rajasthan district of Jodhpur, Jaiselmer and Barmer semiarid areas. Seeds were wrapped in muslin cloth, surface-sterilised in 1% (V/V) mercuric chloride solution for 15–20 min. After rinsing in distilled water they were kept in glass beakers, imbibed for 12 h in distilled water and then were sown in 12 inches of pots filled with 8 kg of sandy and loamy garden soil and proper irrigation was given during experiment. Pots were ploughed to maintain proper aeration and recommended doses of NPK fertilisation (120 : 60 : 60) was applied prior to seed sowing. Pots were kept in open field at photosynthetic active radiation (PAR) in range of 678–768 $\mu mol/m^2/s$ maintained at a temperature of 33.1–34.9°C, at relative humidity between 61–71 RH and wind speed in range of 0–0.7 m/s. Seeds were germinated, seedlings grew and after 40 days plant were transferred in rings under elevated and ambient carbon dioxide enrichment concentrations. Both varieties RGC 1002 and RGC 1066 pots were setup in three replicates under circular fabricated aluminium framework maintained under elevated (Ring 1, Ring 2 and Ring 3) concentration (490 ± 50 ppm) and ambient (Ring 4, Ring 5 and Ring 6) concentration (300 ± 50 ppm). Pure carbon dioxide gas is used in enrichment mixed with pure air. A regulator and a circulating pump were used to inject carbon dioxide into the aluminium fabricated ring chambers. A flow meter was used to adjust carbon dioxide concentration to the target level. Carbon dioxide was supplied for 120 days from 9:00 am to 5:00 pm. This whole FACE setup is computer automated with eight data scanner and wind monitor logger which monitor and capture per day data. Plants grown in the above condition for 160 days (5 months) including seed sowing to seedling stage of 40 days from 25 April 2014 to 30 October 2014 and plant stage with routine and proper irrigation were maintained. Two plants of each variety from three replicates of elevated and ambient ring were harvested for analysis of plant growth and biomass. All plants parts were separated including leaves, stem and roots. Fresh weights of all parts were estimated in grams and total fresh weight is determined by adding individual fresh weight of all parts. Now all the parts were dried in oven maintained at 80°C for determining plant dry weight [21]. Leaf area were recorded using LI-3000C leaf area meter (LICOR, Inc, Lincoln, NE, USA). After 60 days leaves were excised, washed with distilled water soaked with tissue paper, frozen immediately in liquid N_2 and stored at −80°C. The frozen plant sample were grounded to fine powder in a pre-cooled mortar and pestle with liquid nitrogen (N_2) and the samples were stored in 15 mL volume tarson tubes in −80°C for extraction for further enzymatic assays.

2.2. Biochemical Assay Nitrate Reductase, Nitrite Reductase and Glutamate Synthetase Enzyme Extraction and Activity Assays

2.2.1. Nitrate reductase enzyme assay. The Frozen plant material was homogenized ina chilled mortar and pestle with 100 mM potassium phosphate buffer (pH 7.4), containing 7.5 mM cysteine, 1 mM EDTA and 1.5% (w/v) casein. The plant homogenate was centrifuged at 30000 × g for 15 min at 4°C. Nitrate reductase activity (NRA) was determined according to the method of Robin (1979). The extract was incubated in a reaction mixture containing 100 mM potassium phosphate buffer (pH 7.4), 10 mM EDTA, 0.15 mM NADH and 0.1 M KNO_3 at 30°C for 30 min. The reaction was stopped by 100 mL of 1 M zinc acetate. Absorbance of the supernatant was determined at 540 nm after diazotation of nitrite ions with 5.8 mM sulphanilamide and 0.8 mMN-(1-naphthyl)-ethylene diamine dihydrochloride (NNEDD) in UVB-131312 Thermo scientific Spectrophotometer.

2.2.2. Nitrite reductase enzyme assay. Enzyme extracts were prepared as described above for nitrate reductase. Nitrite reductase activity (NiR) was assayed

by the method of Losada and Paneque (1971). The extract was incubated in a solution containing 100 mM potassium phosphate buffer (pH 7.4), 15 mM sodium nitrite, 5 mM methyl viologen, 86.2 mM sodium dithionite in 190 mM $NaHCO_3$. The reaction was stopped by violent agitation on a vortex mixer. Nitrite ion concentrations were measured at 540 nm after diazotation with 5.8 mM sulphanilamide and 0.8 mM NNEDD in UVB-131312 Thermo scientific Spectrophotometer.

2.2.3. Glutamate Synthetase enzyme assay. The activity of glutamate synthetase (GOGAT) were measured as previously described for NADH-GOGAT [22]. Plant samples were crushed in liquid nitrogen and the samples were stored in tarsons and kept at −80°C. Enzyme was extracted in 50 mM potassium phosphate buffer (K_2HPO_4) with EDTA (pH 7.5) and the supernatant is extracted. To assay the enzyme activity 50 mM tris HCl buffer is prepared, 5 mM 2-oxo-glutarate, 5 mM glutamine and 0.2 mL enzyme extract is mixed. The reaction mixture was incubated for 30 min at 37°C. After the completion of reaction 0.25 mM NADPH is added and the rate change is monitored at 340 nm in UVB-131312 Thermo scientific Spectrophotometer.

2.2.4. Estimation of total nitrogen % age in plant and soil samples. Plant and soil total nitrogen % age were determined by Kjehaldal method-Jacson (1962). In this method 1 gm of soil and plant sample were taken and these samples were pre-digested and run in nitrogen aceto analyser on programme 0. Plant and soil sample were pre-digested with 10 mL of conc. H_2SO_4 with mixture of 3.4 gm K_2SO_4 and 0.4 gm $CuSO_4$. The mixture was heated at 200–300°C in digestion block. Standard was prepared with 0.2 gm Ferrous ammonium sulphate (FAS) containing 7% nitrogen.

2.2.5. Statistical analysis. The experiment was conducted in a randomized plan with three replicates of each treatment dose from triplicate rings maintained at elevated and ambient carbon dioxide concentrations. Statistical analysis of the data was done following the methods of analysis of variance (One way Anova) by SPSS 16.0 software version. The standard deviation (SD) values between the treatments were calculated at 5% probability levels and significance of data is analysed ($p \leq 0.05$).

3. RESULTS

3.1. Effect of Elevated CO_2 on Plant Morphological Growth Parameters

Cyamopsis tetragonoloba plants were grown under elevated carbon dioxide (490 ppm) concentrations for 120 days and several morphological parameters were determined. Fresh weight of Plant, Dry weight of Plant and Leaf area/cm^2 (Table 1) of all replicates of two varieties RGC 1002 and RGC 1066 were determined. The result showed that plant exposure to carbon dioxide lead to increase in plant fresh weight, plant dry weight and leaf area/cm^2. Plant total fresh weight was found significantly greater (40%) under carbon dioxide elevation in both the varieties than ambient, while RGC 1002 showed comparatively higher (25%) plant fresh weight than RGC 1066 variety. Similarly total dry weight of plant showed significantly increased trend (25%) in both the plant varieties than ambient with RGC 1002 showed a increased (10%) dry weight than RGC 1066. Plant exposure to elevated carbon dioxide promoted an increase (35%) in leaf area/cm^{-2}. Contrary RGC 1002 showed higher increase in leaf area than RGC 1066 varieties. These results indicate that increase in overall growth parameters is due to high CO_2 effect.

3.2. Effect of Elevated CO_2 on Nitrate Reductase, Nitrite Reductase and Glutamate Synthetase

Activity of nitrate reductase (NR; EC 1.6.6.1), nitrite reductase (NiR; EC 1.7.7.1), glutamate synthetase (GOGAT; EC 1.4.1.13) were analyzed under carbon dioxide elevation in (*Cyamopsis tetragonoloba*) leaves. The NR activity significantly declined in elevated carbon dioxide grown plants despite increased in growth parameters (figure). The overall reduction of NR activity was (25%) in elevated carbon dioxide concentrations when compared with ambient carbon dioxide concentrations. RGC 1066 showed comparatively higher significant (22.24%) decreased in NR activity than (23.07%) in RGC 1002 variety. Thus NR activity also reported a significant decreased under elevated condition when compared with ambient conditions. The decreased in NR activity between RGC-1066 and RGC-1002 varieties is 3.717% under elevated carbon dioxide concentration. The decreased in NiR activity was showing little significant difference (19.02%) in RGC-1002 variety under elevated carbon dioxide concentration when compared will ambient carbon dioxide concentration. Similarly RGC-1066 showed 18.61% decreased in NiR activity under elevation carbon dioxide concentrations and overall decreased was 18.50% in NiR was noticed. The decreased in NiR activity between both the varieties was 3.59% under elevated carbon dioxide concentrations. This decreased in NiR activity may be due to less NO_2^- available for reduction. GOGAT activity overall declined (23.28%) significantly in both the varieties of *Cyamopsis* under elevated carbon dioxide concentrations. The decrease in the activity of Glutamate Synthetase was 33.01% in RGC-1002 variety under elevated carbon dioxide concentration and 13.55% in RGC-1066. Thus the significant difference was noticed in decreased Glutamate Synthetase activity in RGC-1002 when compared with RGC-1066 under elevated carbon dioxide concentrations.

Table 1. Plant morphological growth parameters of two varieties of *Cyamopsis tetragonoloba* (RGC 1002 and RGC 1066) exposed for 120 days under elevated CO_2 (490 ppm) and ambient CO_2 (300 ppm) concentrations. R denotes rings, E denotes Elevation. A denotes Ambient condition. NS Non Significant. * Denotes significant difference. Values are mean ±S.D. ($n = 3$) of triplicate determinations of two separate experiments

PARAMETERS	TOTAL PLANT FWT (gm/plant)		TOTAL PLANT DWT (gm/plant)		LEAF AREA/cm^2 (cm^2/plant)		TOTAL CHLOROPHYLL (CCM unit = μmol/m^2)	
→ VARIETIES RINGS ↓	RGC 1002	RGC 1066	RGC 1002	RGC 1066	RGC 1002	RGC 1066	RGC 1002	RGC 1066
R1E	23.18 ± 1.65(NS)	15.76 ± 2.9^{bc}*	13.65 ± 0.47^{a}	10.62 ± 1.0^{b}*	31.03 ± 2.8^{b}*	45.34 ± 8.54^{a}*	50.33 ± 5.98^{a}	48.3 ± 5.96^{a}
R4A	14.51 ± 0.99(NS)	12.97 ± 2.1^{de}	9.54 ± 1.12^{bc}	8.24 ± 0.30^{d}	21.11 ± 5.7^{d}	30.66 ± 2.8^{b}	28.65 ± 7.15^{de}	27.76 ± 7.07^{e}
R2E	11.44 ± 0.3^{de}	11.54 ± 0.9^{de}*	7.45 ± 0.24^{d}	8.24 ± 0.30^{cd}	30.81 ± 2.8^{b}*	40.72 ± 3.60^{a}*	43.9 ± 7.86^{ab}	44.94 ± 6.46^{ab}
R5A	12.70 ± 1.1^{de}	10.21 ± 0.59^{e}	7.66 ± 0.15^{d}	6.59 ± 0.50	23.57 ± 2.1^{cd}	29.10 ± 7.11^{bc}	33.14 ± 7.15^{cde}	35.22 ± 7.07^{cd}
R3E	21.32 ± 2.83^{a}*	14.44 ± 1.09^{cd}	13.23 ± 1.50^{a}	9.89 ± 0.68^{b}*	30.96 ± 3.1^{b}*	41.31 ± 6.19^{a}*	39.65 ± 7.94^{bc}	44.94 ± 8.66^{ab}
R6A	12.85 ± 1.44^{cde}	17.55 ± 0.66^{b}	7.48 ± 1.21^{d}	7.30 ± 0.21^{d}	25.45 ± 1.82^{bcd}	28.77 ± 4.51^{bc}	35.7 ± 5.79^{cd}	34.98 ± 9.46^{cd}

3.3. Effect of Elevated CO_2 on Plant N, Soil N and Soil pH

The increased in carbon dioxide level significantly affects the total nitrogen content of leaves on dry weight basis in *Cyamopsis* varieties. Elevated carbon dioxide decreases the N content in leaves (Table 2) which may be due to less uptake of nitrogenous compounds from the soil or due to the dilution effect [23] of N in presence of high carbon fixation in growing regions of the plant. Plants grown under elevated carbon dioxide concentrations show overall 12.33%, significant decrease in N content in leaf tissues when compared with ambient carbon dioxide concentrations, in both the varieties. RGC-1002 and RGC-1066 showed a 9.17 and 12.64% respectively declined in total nitrogen content thus not showing a significant difference in two varieties under elevated carbon dioxide concentrations. Total N in soil does not showed a significant change in both elevated and ambient carbon dioxide concentrations (Table 2b) $p \leq 0.05$. Soil pH value showed a significant decrease under elevation which confirms the lowering of pH due to formation of carbonic acid and organic acids in soil via soil biota respiration, roots respiration and diffusion of carbon dioxide gas in soil (Table 2c). Elevated carbon dioxide concentration increases respiration of soil microbes and roots by two fold approximately double below ground carbon dioxide concentration [24]. Each value in the above Fig. 1 represents the average (±S.D) of three replicates. Different lowercase letters indicate significant differences between the elevated and ambient Carbon dioxide concentrations of same varieties. Same lowercase letters do not indicate a significant difference between elevated and ambient carbon dioxide concentrations.

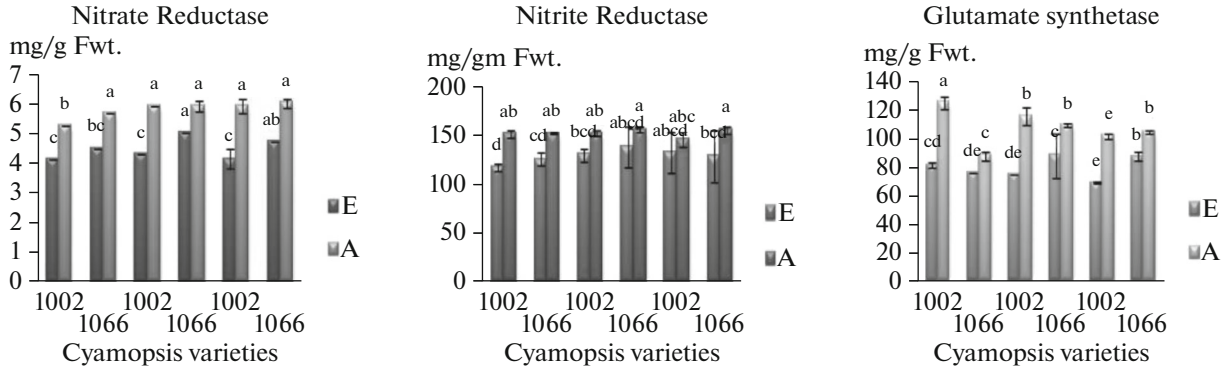

Fig. 1. Nitrate Reductase activity (N.R), Nitrite Reductase activity (NiR) and Glutamate Synthetase (GoGAT) activity in the leaves of *Cyamopsis tetragonoloba* plants exposed to elevated (490 ppm) and ambient (300 ppm) carbon dioxide concentrations. E denotes elevation concentration and A denotes ambient concentration. Error bars represent ±S.D ($n = 2$). CD at 5% between ambient and elevated carbon dioxide levels.

Table 2. Total nitrogen (N) in leaves and soil ((a) total nitrogen in leaves, (b) total nitrogen in soil and (c) soil pH) of *Cyamopsis tetragonoloba* varieties under elevated and ambient CO_2 levels under elevated and ambient condition. Same lower case letters denotes Non significant differences between the parameters. Error bars represent ±S.D. ($n = 2$). CD at 5% between ambient and elevated carbon dioxide levels

PARAMETERS	(a) Total N Plant (mg/g)		(b) Total N Soil (mg/g)		(c) Soil pH (pH units)	
VARIETIES → RINGS ↓	RGC 1002	RGC 1066	RGC 1002	RGC 1066	RGC 1002	RGC 1066
R1E	25.04 ± 1.54^{f}	31.12 ± 2.89^{b}	0.34 ± 0.04^{a}	0.40 ± 0.04^{a}	6.98 ± 0.24^{fgh}	6.84 ± 0.28^{gh}
R4A	30.21 ± 1.76^{c}	32.66 ± 2.45^{b}	0.43 ± 0.03^{a}	0.42 ± 0.03^{a}	7.83 ± 0.26^{abc}	7.53 ± 0.43^{cdef}
R2E	26.44 ± 1.65^{e}	27.87 ± 1.84^{e}	0.37 ± 0.07^{a}	0.33 ± 0.04^{a}	7.36 ± 0.22^{fg}	7.15 ± 0.41^{efg}
R5A	29.65 ± 2.23^{b}	36.43 ± 3.12^{a}	0.42 ± 0.02^{a}	0.45 ± 0.02^{a}	8.39 ± 0.16^{ab}	7.99 ± 0.27^{bc}
R3E	30.55 ± 3.81(NS)	32.12 ± 2.32^{b}	0.37 ± 0.04^{a}	0.38 ± 0.08^{a}	6.48 ± 0.34^{h}	7.60 ± 0.21^{cde}
R6A	33.54 ± 3.43^{b}	35.21 ± 2.87^{a}	0.44 ± 0.04^{a}	0.46 ± 0.04^{a}	7.80 ± 0.33^{cd}	8.68 ± 0.48^{a}

4. DISCUSSION

Cyamopsis tetragonoloba plants were grown under elevated carbon dioxide (490 ppm) and ambient carbon dioxide (300 ppm) concentration for 160 days and various morphological parameters were determined. Plant Fresh weight, Plant Dry weight and Leaf area/cm^2 (Table 1) of three replicates of two varieties RGC 1002 and RGC 1066 were determined. These results showed that plant exposure to increased carbon dioxide (elevation) lead to increase in plant fresh weight, plant dry weight and leaf area/cm^2. This enhancement in growth response in the plant may simply be explained due to the greater allocation of fixed carbon in growing organs of plants. The increased in the assimilation of carbon dioxide (substrate for photosynthesis) is due to increased net photosynthetic rate in C_3 plants, which resulted in higher translocation and the channeling of photo-assimilate (carbohydrates, starch, sucrose etc.) that resulted in increased production of biomass (fodder). The rate of growth depends of no. of active meristematic tissues and the rate of cell division and duration of cell cycle and cell elongation phase. Shortening of cell cycle occurs in root and stem meristematic tissues [25] growth is rapid and fast in younger plant and it declines as plant ages. It causes thickening of stem and root tissues of plant parts with leaf broadening thus enhancing proportional plant biomass. It promoted enhanced root/stem ratio under N limited plants promoting increased nitrogen used efficiency. This similar result was also being reported in earlier studies done on leguminous crop mung bean [16]. The effect of CO_2 elevation caused greater increase in leaf size, leaf no., leaf thickness and effective increase in leaf area/cm^2. Similar study was done in cucumber plant under CO_2 elevation and contrasting results were observed [14]. Plant canopy and stem diameter also increased which causes thickening of stem due to the channeling of carbon assimilates at the apex of meristematic tissues of growing region. In sunflower plants nitrogen uptake abridged by 25% but photosynthetic nitrogen use efficiency enhanced by 50% leading to 115% increased in biomass under elevated concentrations of carbon dioxide. RGC-1002 variety being tolerant to elevated carbon dioxide concentration is showing gain in plant fresh weight which can simply be explained by higher % age decreased in glutamate synthetase activity in leaves tissue thus causing restricted and slow mobilisation of glutamine. More over plant nitrogen also confirms that there is the restricted uptake of nitrate content from soil under elevated carbon dioxide concentrations. Earlier observation showed similar results of increased plant biomass due to increased in leaf number as reported in Japanese honey-suckle [26]. An increased in growth and plant dry weight were also estimated. In addition to increased leaf area/cm^2 carbon dioxide elevation leads to increases in plant dry weight and plant fresh weight in Japanese honey-suckle. Thus total gain in the growth of both varieties under elevated carbon dioxide is the result of increased net photosynthetic rate as observed in many other crop species [27]. Increased net photosynthetic rate also affect the plant nitrogen metabolism due to the generation of the reductants formed by the oxidation of photo-assimilate for nitrate reduction [28].

The Nitrate Reductase activity declined despite of increased carbon dioxide fixation and increased photosynthetic rate. These findings are in favour to the results reported in *Trifolium alexandrinum* (berseem) plant where NR activity decreased under elevated carbon dioxide concentrations [21]. More over similarly decreased foliar NR activity is found in *P. taeda* during carbon dioxide enrichment [29]. The reason for this effect seems to be the competition for the reductant Nicotinamide adenine dinucleotide (NADH) between NO_3^- and carbon assimilation on one hand and inhibition of NO_3^- assimilation on the other hand as found in wheat and Arabidopsis Nitrate solely depends on NADH as reducing power which is the primary requirement for initial step in nitrate absorption [30].

Thus carbon dioxide elevation causes reduction in photorespiration [31] due to feedback inhibition of high carbon dioxide level that reduces the availability of NADH for nitrate reduction which results in decrease in activity of Nitrate reductase and other enzymes of nitrogen metabolism (Fig. 1) as observed in guar varieties. Moreover, elevated carbon dioxide increases N_2O emission from the soil which is the additional cause of N loss that decreases nitrate availability from the soil. These observations are contradictory to the results of earlier studies done by Hocking and Meyer 1991 who observed an increase in NR activity in maize plants grown under elevated condition. Similarly studies done on Cucumber plants reported an increase of NR activity at low nitrate content under elevated condition [14] and increase of NR-gene transcription and NR activation state. Thus it was being inferred that rate of CO_2 fixation controls the NR expression which is up regulated at both transcriptional and post-transcriptional level in short term exposure of cucumber plant to carbon dioxide elevation but plant exposure for long duration behave differently. Carbon dioxide deprivation restrict carbon fixation [32]. Nitrite reductase and glutamate synthetase activity is found declined under elevation as it is linked with decreased in nitrate and NADH availability in plant tissues. This declined in nitrite reductase and glutamate synthetase activity can be due to less translocation of nitrate from the soil or reduced availability of nitrite in plant tissue. The reduction in nitrate uptake by the plant is due to reduction in soil pH under carbon dioxide elevation [33]. The reduction in soil pH causes soil acidosis which leads to reduced translocation of nitrate in plant. More over soil carbon dioxide have various effects on mineral dissolution and acidity. Previous studies reported that major source of soil acidity is the carbon dioxide derived from the respiration of soil microbes, plant roots and diffusion. It reacts with soil moisture and forms carbonic acid [34]. The H^+ and HCO_3^- ion generated causes a lowering in soil pH by contributing proton to soil pool under carbon dioxide elevation. Thus the overall soil pH is reduced because higher atmospheric carbon dioxide concentration [34]. However this proton pool outpaces mineral dissolution because of rapid kinetics of exchangeable surface cations generated from carbonic acid, so mineral dissolution is buffered. Moreover the decreased amount of nitrate in leaves tissue causes less reduction of nitrate to nitrite by nitrate reductase and nitrite to ammonia by nitrite reductase. Inorganic ammonia formed assimilates into glutamine and glutamate via GS/GOGAT which is the primary synthetic nitrogenous compound in higher plants. The primary substrate for ammonia assimilation is plant nitrate which is reduced by NR/NiR. NH_4^+ formed is incorporated in the glutamine amide linkage by Glutamine synthetase, and then into glutamate amino linkage by glutamate synthetase under physiological conditions. GOGAT occurs in two isoforms in plants:ferrodoxin-dependent GOGAT (Fd-GOGAT) and NADH-dependent GOGAT (NADH-GOGAT). Some ammonia produced through nitrogen fixation in root nodules also contribute towards glutamine and glutamate synthesis. GS and GOGAT are key enzymes of ammonia assimilation which is derived from nitrate reduction, N_2 fixation as well as from various secondary metabolic processes for eg. photorespiration or amino acid catabolism. Photorespiration is a major physiological process that additionally releases high amount of ammonia rather than its de novo synthesis [35] which is suppressed by elevated carbon dioxide concentrations. Additionally Photorespiration promotes release of malic acid and availability of NADH (cytoplasmic) required for nitrate reduction and assimilation. This is a reason of decreased in GOGAT activity under carbon dioxide elevation. Thus it is being inferred that although elevated carbon dioxide leads to increase in plant overall biomass and enhanced growth as our results justify, at the same time it also decreases N uptake. Thus N availability is the central factor that limits plant growth parameter but as it is leguminous plant so nitrogen fixation supplements N content thus promoting growth and biomass. Previous studies done on *M. truncatula* and Jemonong plants also showed that growth in plant is limited by N availability [36] under elevated condition. The results of plant total nitrogen suggest that under elevation plant total nitrogen decreases significantly (Table 2a) although no significant changes is reported in soil nitrogen (Table 2b). It has been reported in tobacco and in sunflower [8] that under elevation plant N reduction accelerates early senescence in leaves. The Senescence is due to early immobilisation of reduced nitrogen in plants that causes to decrease in activity of key enzymes (NR, NiR and GOGAT) of nitrogen metabolism.

5. CONCLUSIONS

The conclusion of the experimental study is that the overall effect of CO_2 elevation (490 ppm) on *Cyamopsis tetragonoloba* varieties is analogous to many C_3 plant species. A wide number of literature are in agreement of decline in 10–15% of N^{37} with CO_2 elevations. This suggests that N decrease predominates under elevation over other factors that tend to increase total N. Plant yield is the major factor that must be kept in mind as far as leguminous crop is concerned. Moreover, the nutritional quality of seed is of utmost importance for leguminous crop plant. The present experimental study demonstrate that under elevation the overall biomass of the plant increased significantly, which suggest that fodder production increases 25–35% annually per unit area when compared with ambient grown plant varieties. On the contrary elevated CO_2 is affecting plant NO_3^- and soil nutrient is

important parameter of plant behaviour to CO_2. Nitrogen reduction under elevation seems to affects the nutritional quality of leaf thus affecting seed quality and production. RGC 1002 variety was found to be tolerant to elevated carbon dioxide concentration than RGC 1066 plant variety as our result justify. Moreover, the C : N ratio will increase showing a reduction in nitrogen content and increased in carbon content on mass basis in leaves causes reduction in protein content which deteriote the nutritional quality of seed. Soil nitrogen replenishment phenomenon may also be affected that will contribute less nitrogen to soil and will affect the overall soil fertility. Two parameters need additional investigation that will provide more clear view of physiological changes occurring in plant under elevation for eg. Change in leaf carbohydrate content, macro and micro nutrient dynamic in plant and soil, enzyme cofactor and soluble protein analysis.

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