RESEARCH ARTICLE

Enhancement of Carbon Assimilates and Macronutrients in Legumes under Elevated CO_2 Concentration

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Abstract

Impact of elevated carbon dioxide (Free Air Carbon dioxide Enrichment) was studied on the plant chlorophyll, plant growth, plant macronutrients, total starch, total carbohydrate and the activity of phosphoenol pyruvate carboxylase (Pep C) enzymatic assays in leaves, pods and seeds in leguminous plant *Cyamopsis tetragonoloba*. Plants of *Cyamopsis tetragonoloba* (C_3) were exposed to different atmospheric CO_2 concentrations 420 ± 20 ppm (ambient) and 550 ± 20ppm (elevated). An average increase in the plant total chlorophyll (+39.17%), total starch (+43.73%, +25.44% and +26.35% in leaves, pods, and seeds), sucrose (+69.77%, +22.27% and +33.77% in leaves, pods and seed) and total carbohydrate (+58.88%, +30.54% and +28.38 % in leaves, pods and seeds) content were found in plant grown under elevated condition when compared to ambient counterpart. More over overall plant growth (+40% height and +25% biomass) increased in e[CO_2] concentration. Plant total nitrogen (N) content decreased (−12.55% in leaves) under the elevated condition where as total phosphorus (P) decreased (−3.15% in leaves) along with total potassium (K) (−46.63% in leaves). In soil, total potassium (+60.23%) and phosphorous (+48.88%) were found to increase with (−16%) decrease in soil nitrogen content. In seed total nitrogen content increased (+18.15%) on an average with no significant change in total potassium and phosphorus content under e[CO_2]. Phosphoenolpyruvate carboxylase enzyme (Pep C) (+139.5% in leaves) activity and total organic carbon (TOC) (+19.12%, +17.85% in leaves and seeds) increases in elevated concentration thus promoting and indicating higher photosynthesis via enhanced CO_2 fixation. Thus our studies showed that e[CO_2] positively promotes sugars, carbohydrates synthesis, translocation and partitioning in plant tissues and enhanced macronutrients level in leaves and seeds tissue which is contradictory to other C_3 plants. Thus e[CO_2] works as the boon for *Cyamopsis* varieties and the seeds are nutritionally rich, healthy, balanced in proteins and carbohydrates (C/N) and so these varieties have future implication for industrial use in the agricultural country like India.

Keywords: Pep C, *Cyamopsis tetragonolaba*, NPK, TOC, e[CO_2], a[CO_2].

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Introduction

Plant growth and biomass contrast between elevated atmospheric carbon dioxide (eCO_2) concentration and current ambient concentration (aCO_2) that affects the plant tissue biochemistry. Climate change, plant growth, plant macro and micronutrients, carbohydrates, sugars and their interactive effects are the future challenges for plant biologists and physiologists. The concentration of carbon dioxide is increasing globally at an alarming rate and plant adaptation to changing carbon dioxide concentration is a major scientific problem. Various human anthropogenic activities, industrialization and emission of carbon dioxide, methane, halocarbons (CFCs) and N_2O are the major sources of this abrupt increase in surface warming and climate change (IPCC 2013). Previous studies demonstrated that the concentration of atmospheric carbon dioxide was 280ppm before industrialization in the 19th century and is 400ppm in 2015 and is expected to reach 450ppm-600ppm by the year 2050 (IPCC 2007) and 650ppm to 900ppm by 2100.

Now it is interesting and challenging to understand how the plant will behave and adapt to this rapidly changing carbon dioxide concentration and predicting about their ability to adapt, is the primary step in understanding while keeping in mind about all multiple interacting factors globally and their impact on the ecosystem.

Previous experiments being conducted and earlier studies reveal that although carbon dioxide elevation stimulates and promotes biomass and growth enhancing responses in some leguminous varieties it also showed a neutral effect on some varieties. In the last two decades, it was being summarized by different researchers about the aspects of the main effects of growing legumes at elevated CO_2. Among them, improvements of photosynthetic rates and increases in vegetative growth in short-term experiments are highlighted without emphasizing much on long term exposure basis experimental plans. However, the legume responses to elevated CO_2 are strongly modulated by many other such as excess temperature, water, and nutrient availability, etc. The increased biomass and growth in plant is

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due to increased rate of photosynthesis that results in high yield in many crop varieties grown under an elevated level of carbon dioxide (Das *et al*. 2002). Moreover, under short-term exposure of carbon dioxide concentration rate of photosynthesis increases and ribulose-1,5–biphosphate carboxylase/oxygenase activity is enhanced (Moore *et al*.1999). Long-term studies provide less convincing results of doubling of photosynthetic rate and a decrease in soil moisture content at twice-ambient carbon dioxide concentration than under short term exposure condition and carbon dioxide concentration. Apart from yield, nutritional value of food grains the matter of utmost concern nowadays under changing environment like increasing carbon dioxide concentration. There are presently no studies which provide convincing results of elevated CO_2 effect on seeds nutritional quality and quantity at maturing stage.

Free Air Carbon dioxide Enrichment (FACE) experiments previously conducted provides more than enough evidence that in C_3 plants photosynthetic activity acclimatizes on a long- term basis under carbon dioxide elevation and its down regulation varies with genetic and environmental factors. Recent studies conducted demonstrated that under carbon dioxide elevation photosynthetic carbon uptake (A) is enhanced despite of acclimation of photosynthetic capacity. Photosynthetic acclimation is calculated as a decreased form of Vc, max which denotes the maximum carboxylation rate of Rubisco and Jmax which denotes the maximum electron transport rate leading to ribulose-1,5-bisphosphate (RubP) regeneration. In comparision with the RuBP carboxylase and other PCR cycle enzymes level of PEP C was reported in a much higher amount in leaves, seed, and pods of Cicer arietinum by Singh (1989) under a[CO_2] but least reported are available under e[CO_2]. Photosynthetic acclimation is detectable in the plants by a succession of metabolic changes in leaf total soluble proteins as well as increases in Carbohydrates, Sugars etc. This acclimation of photosynthesis may have important consequences in plant production, limiting the positive effects of elevated CO_2 on plant yield, and also on crop yield quality. (Irigoyen *et al*. 2014) Photosynthetic carbon uptake (A_{sat}) is stimulated in C_3 plants grown at elevated carbon dioxide condition despite the decrease in $V_{c\ max}$, and J_{max}. (Ainsworth *et al*. 2007) but the degree of stimulation of (A_{sat}) varies among species and experimental condition (Ainsworth *et al*. 2005; Nowak *et al*. 2004).

Elevated CO_2 results in accumulation of carbohydrates in plant tissues, as there production exceeds their utilization under these conditions (Moore *et al*.1999; Wolfe *et al*. 1998). Previous studies showed that increased in plant growth, biomass and carbon metabolites are not always supplemented by increased in essential nutrients elements (Conroy 1992; Manderschield *et al*. 1995). Nitrogen, Phosphorus and Potassium (NPK) plays an essential and important role in agricultural crop production. In natural form, the soil is equipped with sufficient quantities of essential elements for plant growth. However due to agricultural crop production soil becomes depleted with these essential elements and require biological soil amendments in order for crop to remain productive. Nitrogen is an integral part of all protein, and important chemical element required for plant growth and photosynthesis. Plant absorbs it in form of either ammonium or nitrate through its root system. Deficiency leads to a yellowing (Chlorosis) of leaves because of declining chlorophyll, symptoms first develop on older leaves than on younger leaves, stunted plant growth, fewer tillers occur in grains, low protein content in seeds and vegetative parts, fewer leaves, and susceptibility to weather, stress, pest, and diseases. Phosphorus plays an array of functions necessary for healthy plant growth including structural strength, crop quality, seeds production. Moreover, it encourages the growth of roots, promotes blooming and essential in DNA synthesis. Insufficient supply can cause green and purple discolouration on leaves or petioles, wilting, small fruits and flowers. Potassium, the third of three elements in healthy soil nutrition can greatly increase crop yield, aids in water absorption and retention, encourages strong roots, sturdy stems and healthy full grown crop with longer self life. Moreover it aides plant in using water efficiently, preventing many disease and damage and help in cycling nutrients through leaves, stem, and roots. Deficiency includes leaves scratching, curling of leaf tips as well as yellowing between the veins. Plant growth, root development, seeds, and fruit are reduced during potassium deficiency. In India Elevated carbon dioxide enrichment studies were conducted under mid FACE system in Trifolium alexandrinum (berseem) it was found that the nutritional value of crop is declined due to reduction in concentration of major macro and micronutrients on per unit mass basis (Pal *et al*. 2004). However, in India, there is hardly any information about the impact of elevated CO_2 on macronutrients, Pep Carboxylase enzymatic assay and Carbohydrates and sugars metabolism in leguminous edible crop.

The principle of our experimental study was to investigate how carbon metabolism is affected in leguminous crop under carbon dioxide elevation with special emphasis on:

- Physiological performances including Photosynthetic rate (PN) and Stomatal Conductance (gs).
- Carbon metabolism enzymes Pep Carboxylase (Pep C) activity.
- Total carbohydrate, Starch, and Sucrose content in leaves, pods, and seeds.
- Macronutrient uptake in the plant, seeds, and soil.

This work is focused on legumes, with special emphasis on guar (*Cyamopsis tetragonoloba*). Guar or cluster bean (*Cyamopsis tetragonoloba* L.) is a well-known drought and salinity tolerant summer annual legume. Guar increases N and organic matter content of soil by atmospheric nitrogen fixation phenomenon and adding plant residues. Cyamopsis tetragonoloba is important at world scale for human nutrition, galactomannan content in seeds in form of guar gum and as forage for animal nutrition. A relatively short growing season (90-120 days) of guar makes it a viable rotation crop. Green guar pods are a rich source of minerals, fibers, protein, and vitamin C. These species represent an excellent opportunity to analyze the effect of elevated CO_2 and climate change in plant vegetative growth. These aspects are important not only at the economic but also at the plant physiological level.

Previously many studies and predictions were made on physiological and ecological parameters and performances yet an urgent need exist to touch and assess the changes in the quantitative and qualitative traits of plant tissues in particular for carbon assimilates and macronutrient concentration under e[CO_2]. Our hypothesis is to evaluate the effect of e[CO_2] on overall growth parameters, biomass and yield parameters,

carbohydrate, sucrose and starch content in leaves, pods and seeds (C assimilates) with the macro- nutrients (nutrient level) content in leaves, pod and seed in *Cyamopsis tetragonoloba* under Free Air Enrichment System (FACE). Moreover, an attempt was made to monitor net photosynthetic rate, stomatal conductance in leaves, leaf area, and other attributes.

Materials and Methods

Plant Materials and Growth Conditions

The experiment 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 (Fig. 1), 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 230-233µScm^{-1}. Seeds of *Cyamopsis tetragonoloba* two varieties RGC 1002 and RGC 1066 were taken from the belt of Rajasthan district of Jodhpur, and Barmer semiarid areas. Seeds were wrapped in muslin cloth, surface- sterilized 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 hrs in distilled water and then were sown in 12 inches of pots filled with 8 kg of sandy and loamy garden soil. Pots were ploughed to maintain proper aeration and recommended doses of 5g/pot NPK fertilization (30 kgNha^{-1}:60 kgPha^{-1}:40 kgKha^{-1}) was applied prior to seed sowing. Three irrigation was applied at a regular interval (2.000 m^3 ha^{-1}) to crop with drip system to avoid the stress condition. Pots were kept in open field and photosynthetic performances were recorded between (9:30am-10:30am) am at photosynthetic active radiation (PAR) in range of 678-768µmol photons m^{-2}s^{-1} solar irradiance, at average temperature 33.06°C in range of (Max.39.8°- Min.26.25°C) (Fig. 2. (a)), at Percentage humidity average value of 59.16 % between (Max. 82.03%- Min.36.28 %) (Fig. 2. (b)), average precipitation 9.33 mm in range between (Max.18.66 mm-Min. 0 mm) (Fig. 2(c)) and wind speed on an average 5.6 km/h in range between (Max.71.12 km/h- Min 0 km/h)(Fig. 2(d)) throughout the experiment. Seeds were germinated, seedlings grew and after 40 days plant were transferred in rings under e[CO_2] and a[CO_2] 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 (550 ± 20 ppm) and ambient (Ring 4, Ring 5 and Ring 6) concentration (420 ± 20 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 and was supplied for 120 days. This whole FACE setup (Fig. 3) is computer automated with eight data scanner and wind monitor logger which monitor and capture per day data. Plants grown in the above condition in sunlight as an illuminating source for 160 days (5 months) including seed sowing to the seedling stage of 40 days from 20 April 2015 to 12 Nov 2015 and plant stage with routine and proper irrigation were maintained.

Plant Growth and Yield Analysis

Phenotypic characterization of both plant varieties e[CO_2] and a[CO_2] has been shown in Fig. 4 (a-d). The Plant was harvested for growth parameters and biomass analysis at 60 days (pre- flowering) and 120 days (post-flowering) exposure

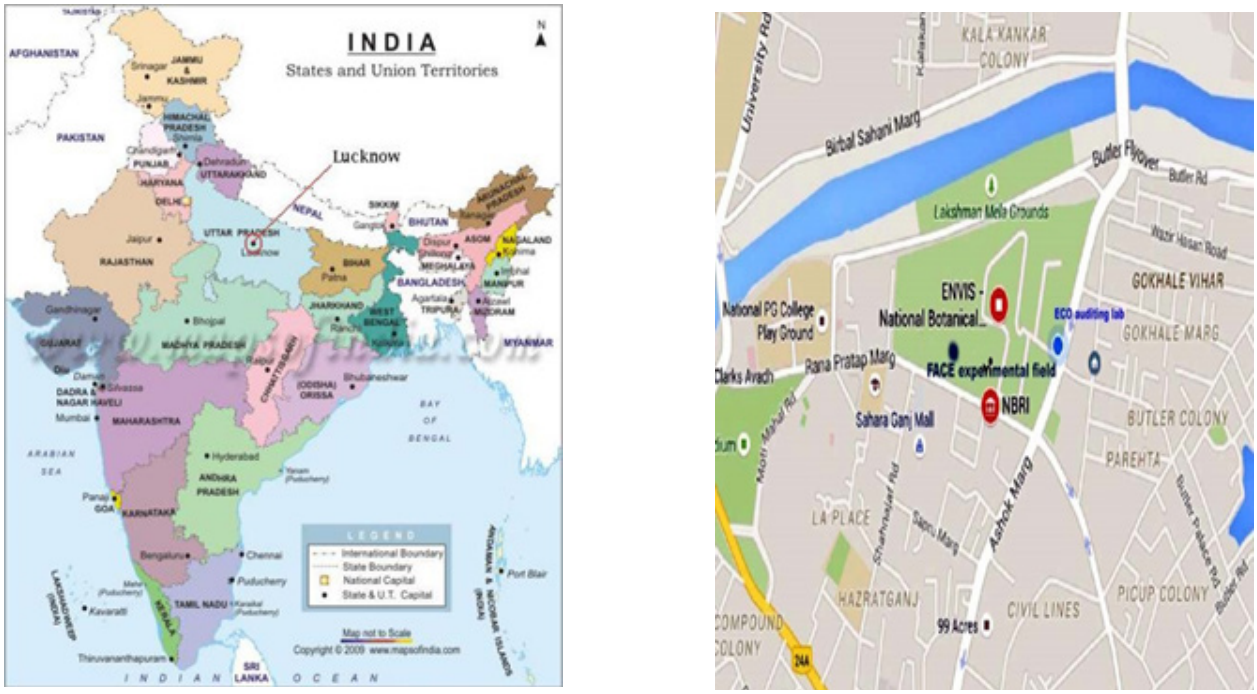

Fig. 1: 1st image shows location of Lucknow in INDIA map and 2nd shows FACE experimental setup in Lucknow, INDIA (Image reference- 1st www.mapsofindia.com, 2nd https://www.google.co.in/maps/@26.8445023,80.937275,14).

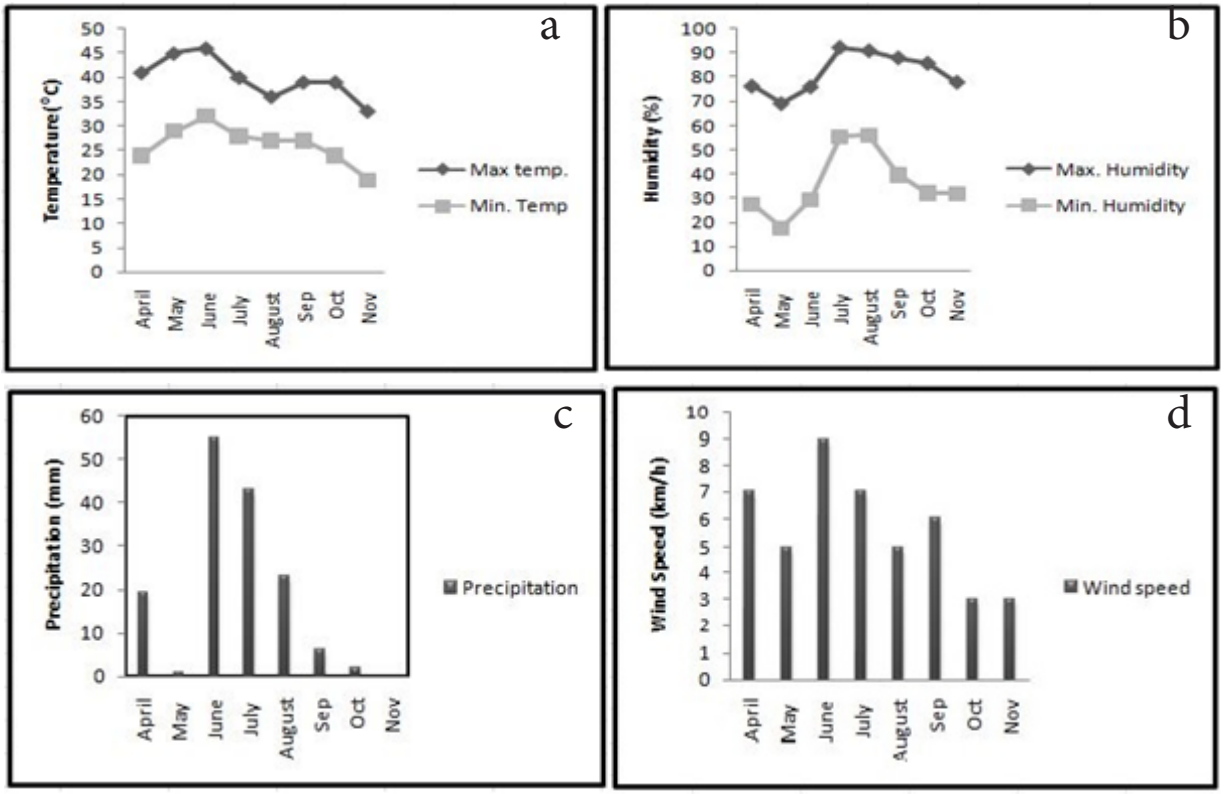

Fig. 2: Maximum and Minimum Temperature, (a) Humidity (b) Average Precipitation and (c) Average Wind Speed (d) in 2015 at FACE Experimental Site N.B.R.I Lucknow.

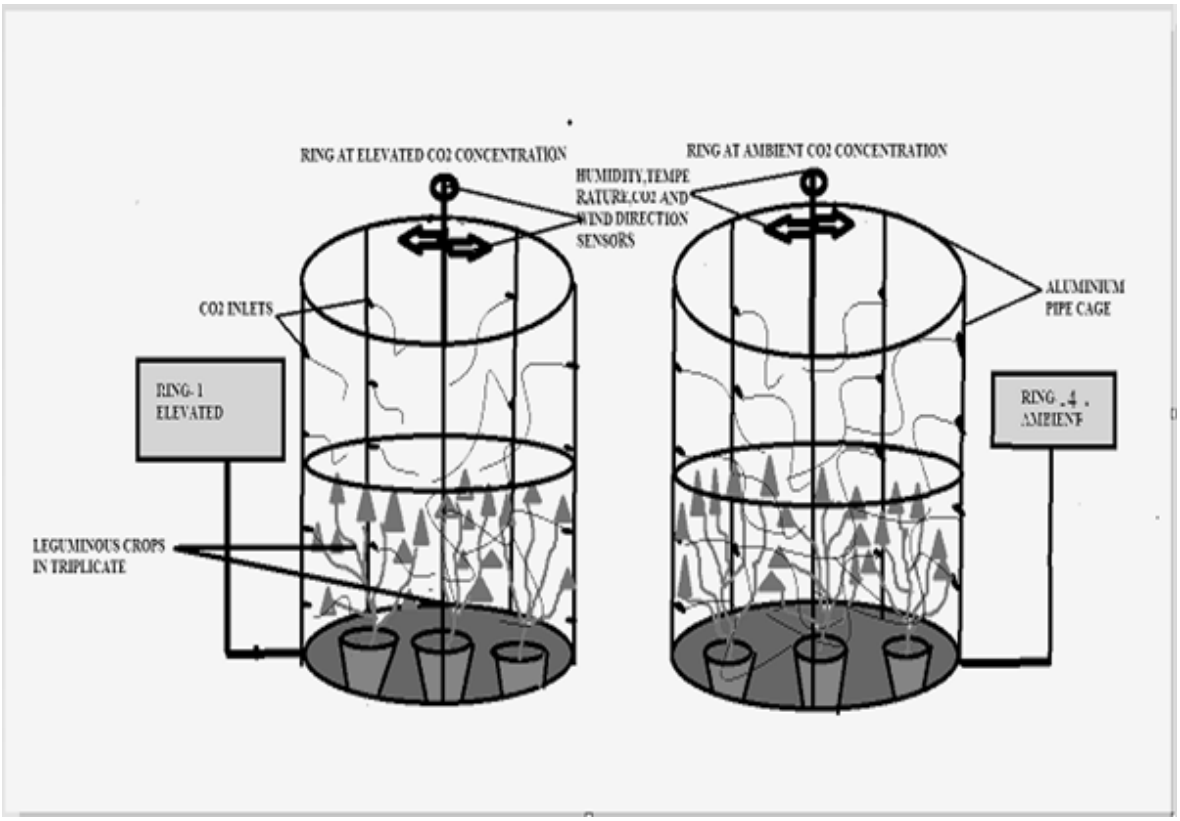

Fig. 3: Pictorial Representation of the FACE setup with Rings maintained at Elevated Carbon dioxide concentrations (RING 1,2,3) and Rings maintained at Ambient Carbon dioxide concentrations (RING 4,5,6) with Carbon dioxide,Humidity,Temperature and Wind direction monitoring sensors.

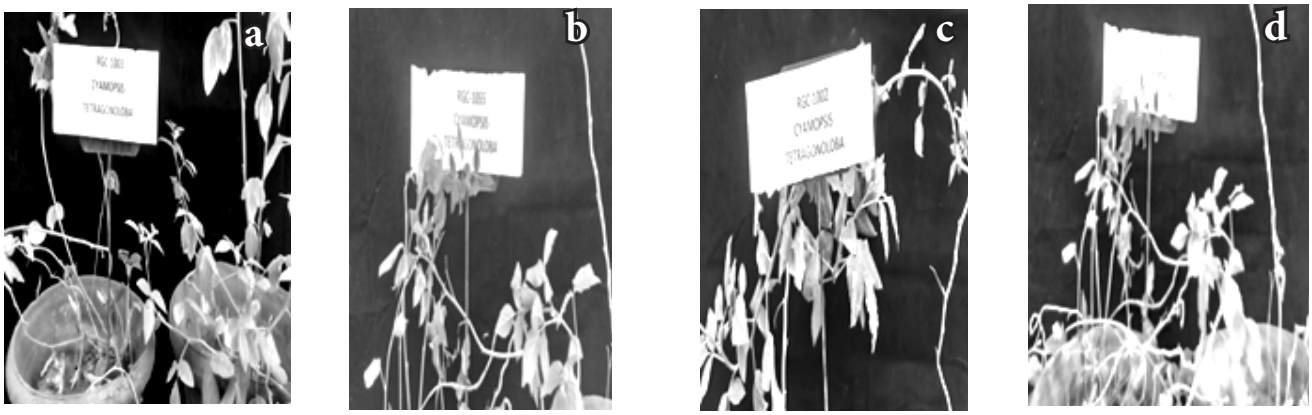

Fig. 4: Phenotypic characterisation of plants varieties (a) RGC-1002 grown at e[CO_2] concentration, (b) RGC-1002 grown at a[CO_2] concentration, (c) RGC-1066 grown at e[CO_2] concentration, (d) RGC-1066 grown at a[CO_2] concentration.

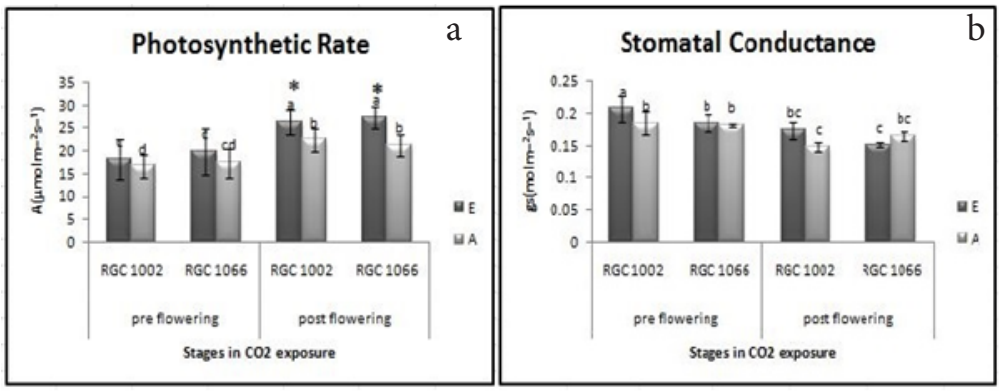

Fig. 5: Photosynthetic rate (a) and Stomatal conductance (b) in both the varieties at pre and post flowering stages of *Cyamopsis tetragonoloba* (RGC 1002 and RGC 1066) in leaves exposed under e[CO_2]=550 ± 20ppm and a[CO_2]=420 ± 20ppm.E denotes elevated and A denotes ambient concentrations. Values of different lower case letters showed significance difference in varieties by turkey post hoc test at 5% probability level. (*) denotes level of significance at p<=0.05.Error bars represents ± S.D (n=9) probability at 5% significance level.

to plant under e[CO_2] concentration. Total plant chlorophyll of both the varieties is recorded by CCM-200 PLUS Chlorophyll content meter. The data is recorded in triplicate including ten observations per plant. Leaves of each stage are included in recording total chlorophyll. Similarly, leaf blade area was measured using a LICOR LI-3000C (LI-COR, Inc., Lincoln, NE, USA) in triplicate including ten observations per plant. Plant morphological parameters include plant height, plant no. per pod and yield per plant is determined by analyzing seeds no. per pods and seed weight. The root, leaves and stem portion were separated, collected and orations were dried at 80°C in oven for determining plant dry weight. Similarly, root/stem fresh weight and root/stem dry weight were also determined. After 60 days and 120 days, plants 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 was grounded to a 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 analysis of enzymatic assays.

Measurement of Physiological Parameters

Net Photosynthetic rate (PN) and Stomatal Conductance (gs) was measured on three plants per treatment using portable photosynthetic system (LICOR, USA) model 6400 during 9:30-10:30 h of the day. Net Photosynthetic rate were monitored and recorded in pre and post flowering stages in each plant for which three upper expanded leaf was selected under saturating photon flux density of 1200 $\mu molm^{-2}s^{-1}$ was supplied from LED light source and equipped with CO_2 control module.

Biochemical Analysis

Phosphoenol Pyruvate Enzymatic Assay

The frozen plant sample stored at -80°C was grounded in pre-chilled motor and pestle in liquid nitrogen .The sample was homogenised in pre-chilled motor and pestle with 0.1M Tris HCl Buffer (pH 7.8) with 10μM $MgCl_2$, 10mM $NaHCO_3$, 5mM phosphoenolpyruvate carboxylase (PEP C) (Ashton *et al.,* 1990). The enzyme extract was centrifuged at 3000g for 20min at 5°C with tris Hcl (pH 8.0) with 50mM MgCl, 5mM Mercaptoethanol

Table 1: Plant growth and biomass parameters of pre-flowering and post flowering stages of two varieties of *Cyamopsis tetragonoloba* (RGC 1002 and RGC 1066) exposed for 120 days under e[CO_2](550 ± 20ppm) and a[CO_2](420 ± 20ppm) concentration.

	PARAMETER	PLANT HEIGHT (cm)	R/S FRSH WT. RATIO	R/S DRYWT. RATIO	LEAF NO./ PLANT	PODS NO./ PLANT	PODS WEIGHT (g)	SEEDS NO./ PLANT	TOTAL CHLOROPHYLL (SPADS unit)	LEAF AREA/ PLANT (cm^2/plant)
PRE FLOWERING	e[CO_2]1002	29.22 ± 2.46^{a}	0.58 ± 0.05^{a}	0.62 ± 0.55^{ab}	13.56 ± 2.52^{b}	–	–	–	25.26 ± 2.30^{a}	18.21 ± 2.48^{b}
	a[CO_2]1002	26.56 ± 1.90^{c}	0.20 ± 0.08^{a}	0.55 ± 0.26^{abc}	9.78 ± 1.35^{cd}	–	–	–	17.40 ± 2.63^{ab}	8.60 ± 0.36^{c}
	e[CO_2]1066	25.89 ± 1.26^{b}	0.21 ± 0.04^{a}	0.47 ± 0.24^{a}	18.89 ± 4.14^{a}	–	–	–	39.28 ± 7.59^{a}	20.49 ± 2.52^{a}
	a[CO_2]1066	20.33 ± 0.88^{c}	0.12 ± 0.02^{a}	0.27 ± 0.13^{bc}	13.33 ± 5.61^{bcd}	–	–	–	16.28 ± 2.85^{ab}	10.46 ± 1.31^{b}
POST FLOWERING	e[CO_2]1002	34.67 ± 5.57^{b}	0.36 ± 0.15^{a}	1.06 ± 0.61^{abc}	20.67 ± 3.28^{cd}	14.00 ± 0.88^{d}	0.30 ± 0.04^{b}	84.00 ± 5.29^{a}	44.63 ± 5.38^{bc}	30.93 ± 0.11^{c}
	a[CO_2]1002	27.11 ± 1.35^{b}	0.15 ± 0.18^{a}	0.75 ± 0.14^{bc}	11.89 ± 4.02^{d}	9.11 ± 1.02^{d}	0.22 ± 0.05^{a}	54.67 ± 6.11^{b}	32.50 ± 3.57^{c}	23.38 ± 2.18^{d}
	e[CO_2]1066	30.11 ± 0.51^{b}	0.46 ± 0.12^{a}	1.22 ± 0.18^{bc}	29.22 ± 3.98^{bc}	9.56 ± 1.39^{d}	0.40 ± 0.04^{c}	57.33 ± 8.33^{b}	46.73 ± 1.69^{ab}	42.46 ± 2.52^{c}
	a[CO_2]1066	21.67 ± 0.88^{c}	0.45 ± 0.13^{a}	0.52 ± 0.10^{c}	14.22 ± 3.15^{cd}	5.78 ± 1.64^{d}	0.20 ± 0.03^{c}	34.67 ± 9.87^{c}	32.65 ± 4.24^{bc}	29.51 ± 1.01^{d}

'e' denotes elevated, 'a' denotes ambient concentrations. Value are mean ± S.D (n=9) of triplicate determination of two separate experiments.<–> denotes not measured. Same lower case letters denotes non-significant differences between the variables at 5% probability ($p \leq 0.05$) level.

and 1mM EDTA. NADH is added as an reaction initiator finally, mixed well and initial rate change was recorded fir 1min. quickly at 340nm in UVB-131312 Thermo scientific Spectrophotometer.

Total Carbohydrate Concentration

Total carbohydrate in the fresh leaf sample is estimated by the Phenol Sulphuric acid method. In this method 10 mg of fresh leaf sample is hydrolyzed by keeping it in a boiling water bath for 3 hours with 0.5 mL of 2.5N HCl. The mixture is cooled to room temperature. Now the whole mixture is neutralized with solid sodium carbonate until effervescence ceases. Volume is maintained to 10 mL with distilled water. The mixture is centrifuged at 10,000 rpm for 10 min. Now take 0.2 mL of supernatant add 0.8ml of distilled water, 1.0 mL of freshly prepared 5% phenol and 5 mL of 96% H_2SO_4 and shake well. Incubate the mixture for 10 min. After 10min shake the content in the tube and place it in the water bath at 25º–35ºC for 20 min. Mix and cool the mixture at room temperature. Record the O.D at 490 nm.

Total Starch and Sucrose Concentration

Fresh and frozen plant material (20 mg) of Cyamopsis leaves and pods were grinded in pre-cooled motor and pestle. Grinded sample were incubated in covered test tubes with 50mM perchloric acid (4.0 mL) at 96°C for 3 min. The extract was cooled, centrifuged, the pellet washed with cold 50mM perchloric acid (1mL) and re-centrifuged. The supernatant were collected in testube and made to volume of 10mL with distilled water for starch determination and free glucose and fructose determination.

a. Starch Determination

Protease (2.5 mg/mL) was dissolved in 0.2M Tris, 0.1M NaCl (pH 7.2) and self-digested for 30 min at 30°C. The extract (Dialysed malt extracts) (250 µL) were incubated (Ahluwalia *et al.,* 1984) with 200 µL of the protease and 200 µL of 0.2M Tris (pH 7.2) at 30°C for 30 min. After this incubation period the digests were boiled for 5 min. to inactivate the protease. Aliquotes (100µl) from the protease digest were made to 1.0-mL volume with sodium acetate buffer (0.2 M, pH 4.8). AMG (2.8U) was added and the mixture incubated at 55°C for 40min. The glucose released by hydrolysis of starch was estimated as before, in duplicate, using hexokinase/glucose-6-phosphate dehydrogenase. The results were expressed as percentage starch of dry weight.

b. Free Glucose and Fructose Determination

Undialysed malt extract (100 µL) were incubated with sodium acetate buffer pH 4.8 (900 µL) at 55°C for 2 hours (as for starch determination). Fructose and glucose were measured using (Ahluwalia *et al*., 1984) hexokinase/G-6-phosphate dehydrogenase with and without the addition of phosphoglucoisomerase, respectively.

Percentage Nitrogen and Carbon Concentration

Total nitrogen and carbon in plant and soil sample is determined in by high temperature combustion method by Analytic Jena multi N/C series TOC/TNb analysers. In this method 0.1mg of sample is burned to ashes form (combustion) with the gas

Table 2: Total Nitrogen (N), Phosphorus (P) and Potassium (K) in Soil and leaves (Total nitrogen in soil, total phosphorus in soil, total potassium in soil and total nitrogen in leave , total phosphorus in leaves and total potassium in leaves) of *Cyamopsis tetragonoloba* varieties under e[CO_2] and a[CO_2] concentrations.

PARAMETERS→ VARIETIES↓	*N SOIL (µg/g)*	*P SOIL (µg/g)*	*K SOIL (µg/g)*	*N LEAVES (µg/g)*	*P LEAVES (µg/g)*	*K LEAVES (µg/g)*
e[CO_2]1002	0.36 ± 0.02^{b}	2606.69 ± 445.94^{a}	6501.67 ± 453.93^{a}	27.34 ± 2.86^{b}	1091.57 ± 66.04^{b}	11151.11 ± 4309.86^{b}
a[CO_2]1002	0.43 ± 0.01^{a}	1656.23 ± 283.72^{b}	4625.00 ± 534.89^{b}	31.13 ± 2.10^{ab}	1144.08 ± 50.67^{b}	23624.44 ± 9004.22^{a}
e[CO_2]1066	0.37 ± 0.03^{b}	2588.01 ± 381.57^{a}	6433.89 ± 774.50^{a}	30.37 ± 2.22^{ab}	1981.05 ± 351.50^{a}	$13804.44 \pm 2278.56^{ab}$
a[CO_2]1066	0.44 ± 0.02^{a}	1586.77 ± 243.02^{b}	4090.00 ± 563.25^{b}	34.77 ± 1.92^{a}	1949.17 ± 308.76^{a}	$17223.33 \pm 2485.99^{ab}$

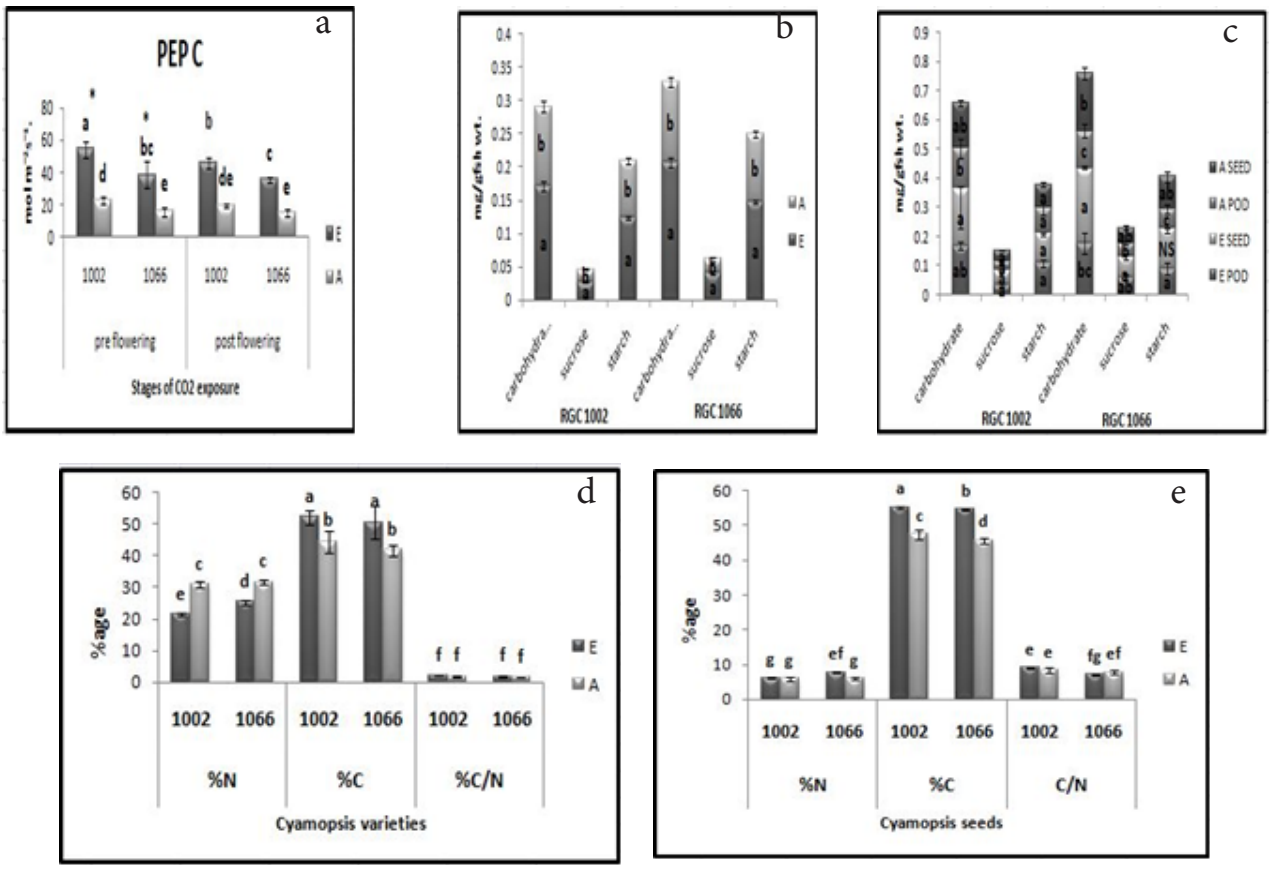

Fig. 6: Phosphoenol pyruvate carboxylase activity (Pep C) in pre and post flowering stages in leaves (a), Total carbohydrate, sucrose and starch in both the varieties in leaves (b), seeds and pods (c), %N, %C,C/N ratio in leaves (d) and %N,%C,C/N in seeds (e) in *Cyamopsis tetragonoloba* plants varieties (RGC-1002 and RGC-1066) exposed under e[CO2] =550±20ppm and a[CO2]=420±20ppm. E denotes elevated, A denotes ambient concentration. Values of different lower case letters showed significance difference in varieties by turkey post hoc test at 5% probability level. Error bars represents ±S.D (n=9) probability at 5% significance level.

source. Generally, sample is composted or pyrolysed and desired gas species usually N_2 or CO_2 is purified by means of traps, filters. The standard gas is measured before and after the sample measurement. This instrument operates by ionizing the sample of interest, accelerating it over the potential difference in kilovolt and separating the streams ions according to their mass-to -charge ratio (m/z).

Estimation of NPK

Total nitrogen in plant and soil sample is determined by Kjeldahl Nitro-analyzer with 40% alkaline NaOH and 1% Boric Acid. Plant and Soil sample (1gm) is digested with 10 mL Conc. HCl, 3.4 gm K_2SO_4 and 0.4 gm $CuSO_4$. The mixture is digested in digestion unit maintained at the temperature of 200-300°C. Programme 1 is set on analyzer. Total Phosphorus in plant and soil sample is determined by digesting 0.1 gm of sample with 5 mL HNO3 and 1 mL perchloric acid. The whole mixture is digested at 170°C until white residue is obtained. The mixture is filtered and the volume is makeup to 50 mL. Filtered solution (25 mL) is mixed with a 1 mL Ammonium molyb date solution and 5 drops of $SnCl_2$ which gives blue colour and O.D is taken at 690 nm. Total potassium is estimated by Flame photometer 128 manufacturer Systronics.

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 analyzed ($p \leq 0.05$).

Results

Plant Growth and Yield

Plant growth was estimated by measuring plant height, root/shoot fresh weight ratio, root/shoot dry weight ratio leaf no./plant and leaf area/plant during pre flowering and post flowering stages. Growth parameters show a significant differences in both RGC 1002 and RGC 1066 cultivars in pre and post flowering stages. Plant height in RGC 1002 increased significantly to 10.01% in pre to 27.11% in post-flowering stage where as in RGC 1066 it increased to 27.34% in pre to 38.94% in post flowering stages (Table 1.). Root/stem fresh weight and dry weight in RGC 1002 increased to 190% and 12.72 % in pre and 140% and 41.33% in post flowering where as in RGC 1006 it increased to 75% and 74.07% in pre to 2.2% and 134.61% in post flowering stages. Similarly leaf no./plant and leaf area/plant in RGC 1002 increased significantly to 38.65% and 111.74% in pre and to 73.84% and 32.29% in post flowering whereas RGC 1066 reported an increase in 41.71% and 95.88% in pre to 105.48% and 43.88% in post flowering stages. Yield study was estimated by measuring pods no./plant, pods weight and seed no./plant. Yield parameters were affected significantly under e[CO_2] concentration. Plant no./pod, pod weight and seed no./plant in RGC 1002 increased to 53.67%, 36.36% and 53.64% whereas to 65.39%, 100% and 65% in RGC 1066 in post flowering stages after final harvesting.

Physiological Parameters and Chlorophyll Estimation

Net Photosynthetic rate, Stomatal Conductance and total chlorophyll content were measured to study physiological plant performances under e[CO_2] concentration in pre and post flowering stages. Net photosynthetic rate was found to increased significantly in both the plant cultivars (Fig. 5. (a)) under e[CO_2] concentration when compared to a[CO_2] concentration. Net photosynthetic rate in RGC 1002 increased to 9.33% in pre to 17.45% in post flowering stages where as in RGC 1006 it increased to 14.08% in pre to 29.17% in post flowering stages. Stomatal Conductances was found in RGC 1002 to increase to 11.89% in pre to 17.21% in post flowering stages (Fig. 5. (b)) whereas in RGC 1066 it does not iincreasesignificantly to 1.93% in pre and it declined to 8.50% in post flowering stages. Total Chlorophyll can be correlated with net photosynthetic rate and it increased abruptly in both pre and post flowering stages. Total Chlorophyll were found to increased significantly (table 1.) in RGC 1002 to 45.17% in pre to 37.32% in post flowering stages however in RGC 1066 it increased to 141.27% in pre to 43.12% in post-flowering stages.

Phosphoenolpyruvate Carboxylase (Pep C) Activity

Phosphoenolpyruvate carboxylase enzyme activity in both the varieties of Cyamopsis varieties was found to be significantly higher in e[CO_2] concentration when compared with the a[CO_2] concentration. RGC 1002 plant variety showed an increase in phospho enol pyruvate carboxylase enzyme activity of 144% increase (Fig. 6. (a)) in pre to 132.29% in post flowering stages when compared with ambient grown plant however its activity declined in post flowering stages. Similarly RGC 1066 plant variety showed an increase of 142.42% in pre to 139.25% in post flowering stages in phosphoenolpyruvate carboxylase enzyme activity under e[CO_2] concentration when compared with a[CO_2] concentration but it also follow similar decline trend in post flowering stages.

Total Carbohydrate Estimation

Total carbohydrate content in leaf samples of Cyamopsis varieties was found to be significantly higher in leaves exposed to e[CO_2] concentrations. RGC 1066 plant variety showed an increase of 74.21% increase in total carbohydrate (Fig. 6. (b)) content when compared with ambient grown plant. Similarly RGC 1002 plant variety showed an increase of 43.55% when compared with ambient grown plants. Overall RGC 1006 showed 30.65% increased in total carbohydrates when compared with RGC 1002 plant variety under e[CO_2] concentrations. Pods and Seeds of RGC 1002 variety showed 23.80% and 26.85% increment in total carbohydrate (Fig. 6. (c)) content under e[CO_2] concentration and RGC 1066 showed 37.29% and 29.91% increment when compared with a[CO_2] concentrations in both the varieties.

Total Starch and Sucrose Estimation

Total starch content in leaf samples of Cyamopsis varieties was found to have increased in plant varieties exposed to e[CO_2] concentration conditions. RGC 1002 variety showed an increase in 44.12% starch concentration (Fig. 6. (b)) under e[CO_2] concentrations were as RGC 1066 variety showed 43.34% increment in starch concentration under the above mentioned conditions .More over RGC 1002 plant variety showed 0.77% increment in starch concentration when compared with RGC 1066 plant variety under e[CO_2] concentrations. Sucrose concentration showed a significant increase in RGC 1002 plant variety with an increase of 48.58% than RGC 1066 showing abrupt increment of 90.97% under e[CO_2] concentrations. Moreover RGC 1002 Pods and seeds showed an increment of 14.89% and 27.80% of sucrose and 22.26% and 29.53% in starch content while RGC 1066 Pods and seed showed an increment of 29.66% and 39.75% of sucrose and 28.63 and 23.16% of starch (Fig. 6. (c)) in both the plant varieties.

%N and %C Content

Total Nitrogen content in leaves declined under e[CO_2] concentration exposed plants in both the varieties (Fig. 6. (d)) with RGC 1002 showed a decline of 30.50% followed by about 20.24% in RGC 1066. Over all RGC 1066 showed 10% less decline in nitrogen than RGC 1002 plant varieties. Total Carbon content improved in both the varieties with RGC 1002 showing 17.29% and RGC 1066 showing 20.96% in comparison to their ambient counterpart. More over C/N ratio increased 3.94% in RGC 1066

and 22.49% in RGC 1002 plant varieties when compared with ambient-grown plants. Moreover, % N results were contradictory in seeds (Fig. 6. (e)) showing 6.45% increment in RGC 1002 and 29.85% increment in RGC 1066 varieties under e[CO_2] concentration. Similarly % C improved to 16.30% in RGC 1002 and 19.37% in RGC 1066 thus C/N ratio approaches to 8.27% increment in RGC 1002 and non significant differences in RGC 1066 plant varieties under e[CO_2] concentration when compared with their ambient counterpart.

NPK Analysis

Total Nitrogen, Phosphorus and Potassium content were estimated in soil with overall 16.00% nitrogen declined (table 2.) under e[CO_2] concentration with no substantial differences between RGC 1002 and RGC 1066 plant varieties. Phosphorus content was found to increase to 57.38% in RGC 1002 soil than RGC 1066 soil showing 63.09%. Moreover, RGC1002 showed 40.47% and RGC 1066 showed 57.30% more Potassium when compared to the ambient counterpart of soil. Nitrogen content declined in RGC 1002 leaves to 12.86% and in RGC 1066 leaves by 12.65%. Leaves Potassium content declined to 52.79% in RGC 1002 and to 19.85% in RGC 1066 plant varieties exposed to elevated concentrations. Phosphorus content declined to 4.58% in RGC 1002 and increased to 1.63%, thus showing no significant changes to ambient concentrations.

Discussions

Effect of e[CO_2] on Plant Growth, Biomass Characters and Yield

Plant varieties exposed under elevated carbon dioxide concentrations results in significant differences in above ground overall growth (table 1.). Plant height increased exponentially under e[CO_2] and a[CO_2] concentrations during the period of growth but elevated carbon dioxide exposed plant were significantly taller in comparison to ambient grown plant varieties. However, RGC 1002 cultivar reported a maximum plant height then RGC 1066 in both pre and post-flowering stages however the difference was more marked in post flowering stages under e[CO_2] concentration. During pre-flowering, the growth was slow and steady but on long-term exposure, it increased at pace. Long-term exposure of Cyamopsis cultivars to e[CO_2] resulted in significant growth enhancement that occurs throughout the period of elevated carbon dioxide exposure. This increase in growth may be due to greater allocation of carbon assimilation under e[CO_2] and that extra carbon fixed by plant gets translocated to growing axis (Sharma *et al*., 1990) Leaf number and leaf area/cm^2 was also significantly greater under e[CO_2] concentration than ambient concentration grown plants. RGC 1066 reported maximum leaf number and significantly higher leaf area/cm^2 than RGC 1002 cultivar under e[CO_2] concentration. In pre and post-flowering, stages significant differences in leaf number and leaf area were reported. In pre-flowering stages, leaf expansion was less pronounced but growth almost doubles in post flowering stages (cure at al.1989) under e[CO_2] than ambient-grown plants. It can be inferred that e[CO_2] stimulates leaf ploriferation and leaf number per plant. Leaf number per plant increase may also be followed by increase in leaf area/cm2 resulting in higher carbon assimilation at ecosystem level as reported in Pinus sylvestris seedling exposed to e[CO_2] by Jack and Ceulemans (1999) in which they predicted that increase in leaf area would result in more rapid canopy closure that may affect on substantial light interception. Plant biomass also reported a increased however it was more in RGC 1066 than RGC 1002. Root /Shoot dry weight ratio is a tool for determining preferential C allocations to root or sprout. Moreover root/shoot ratio increased in both cultivars under e[CO_2] in both pre and post-flowering stages. However, it was highest and almost double in RGC 1066 than RGC 1002 cultivar during post flowering stages below the elevated concentration depicting that growth in the root is more in comparison to the shoot that occurred throughout the period of exposure and justifying that greater C translocation is in the root (sink) than in the radical. Moreover, this experiment demonstrated root length, base number, stem diameter, and root branching accelerate under elevated exposed plants and similar effects were observed in groundnut by (Pilumwong *et al*., 2007).

Various previous study data justify that 10-40% of net C assimilated g*et al*located to below ground biomass (Liljeroth *et al*.1990) that significantly increased under environmental stress and increased carbon dioxide concentration and similar findings were found in e[CO_2] exposed soybean (Allen *et al*., 1988) plants. These results support our findings of increased leaf area in wheat (Pal *et al*. 2005) and it gives an indication of plant activeness in photosynthesis (Fig. 5. (a)) due to effective increased in the leaf surface area and leaf number under e[CO_2] concentrations. Moreover, e[CO_2] has a positive effect on stem weight and leaf number per plant, but comparatively increased in root weight and leaf number contributes to enhanced plant biomass. Leaf numbers accelerate under e[CO_2] with stem weight, which resulted in total biomass. Yield parameters were significantly affected under e[CO_2] concentrations. Pods/plant were higher under e[CO_2] and seed/plant was higher in both plant varieties but on a comparison, it was estimated higher in RGC 1066 than RGC 1002 under e[CO_2] concentration. Thus, overall yield was significantly higher in RGC 1066 plant varieties than RGC 1002 under e[CO_2] concentration exposed plants.

Effe*t* of e[CO_2] on Chlorophyll and Physiological Parameters

Leaf chlorophyll concentration is an indication of the photosynthetic ability of plants and it got enhanced two fold in both pre and post flowering stages under e[CO_2] than ambient-grown plants. The accumulation of photosynthetic pigment was influenced under e[CO_2] depicting enhanced accumulation of photosynthetic pigment including Chl a, Chl b and total chlorophyll. RGC 1066 cultivar reported greater chlorophyll content synthesis in pre- flowering stages than RGC 1002 however in post flowering stages no significant differences were noticed between both the cultivars. Total chlorophyll enhanced in both but it was more in RGC 1066 than RGC 1002 plant varieties. Enhanced level of chlorophyll (table 1.) and photosynthetic pigments provides justification of photosynthetic potential of plant varieties, increased in net photosynthetic rate (Fig. 5. (a)) justifying the results as e[CO_2] grown plants can efficiently capture the photons for

photosynthesis than ambient grown cultivars and so it resulted in increased primary production (Filella *et. al.,* 1995). Previous studies on soyabean (Allen *et al*., 1996) demonstrated that high photosynthetic rate under e[CO_2] is contributed by strong stem and leaf during vegetative and strong seed sink during the reproductive stage. Plants lacking these capacities either inheritically or due to growth in limiting environment tends to demonstrate down-regulation of photosynthetic rate. There was a substantial variation reported between both the cultivars in the extent and amount of photosynthetic pigment and photosynthetic rate (1. and.3.(a)) under e[CO_2] grown plants. Photosynthetic rate (PN) increased effectively in both the cultivars under e[CO_2] than ambient grown plants. The changes in PN under e[CO_2] are often correlated with the changes in Ribulose-1,5 biphosphate Carboxylase/Oxygenase content (Stitt1986) or its activity or regeneration capacity (Sage 1994). The PN was found high in e[CO_2] exposed cultivars than ambient grown cultivars however it increased in both pre and post-flowering stages during the exposed period but no significant differences was noticed in both cultivars under e[CO_2]. This increased in PN during growth period can be simply explained as transient increased of photosynthesis as a stress (Lichtenthaler 1996) response under e[CO_2]. Leaf total chlorophyll and leaf area are also important parameters in determining photosynthetic efficiency so higher total chlorophyll content and more leaf area may also be considered as the chief cause of increased photosynthetic rate under e[CO_2] concentration grown plants. Stomatal conductance is the most importance phenomenon sensitive to e[CO_2] that resulted in partial stomatal closure. Stomatal conductance (gs) increased and was relatively higher in e[CO_2] grown plants than ambient grown plants. In pre, flowering stages gs was significantly higher in RGC 1002 and it subsequently declined in post flowering stages under e[CO_2]. RGC 1066 showed no significant change in gs in pre flowering stages however in post flowering gs declined compared to ambient grown plants. (Ainsworth *et al*., 2002) also reported similar result in soyabean under different e[CO_2] exposures that resulted in declining in stomatal conductance. This can simply be explained by a negative feedback mechanism of the plant to increased e[CO_2] to optimize water loss by adjusting (Medlyn *et al*. 2011) stomatal conductance. Stomatal conductance is the measure of exchange of CO_2 in leaf tissue with water vapour as during day stomatal gaurd cells opens facilitating transpiration in exchange of CO_2. Stomatal conductance is directly related to net CO_2 asssimilation rate which itself depends on e[CO_2] concentration. On long term e[CO_2] exposure a negative feedback mechanism works to conteract the effect of change in e[CO_2] concentration. Stomatal conductance was widely observed (Ainsworth and Rogers 2007) to decrease under e[CO_2] concentration or it increased under decreased in a[CO_2] concentration.

Effect of e[CO_2] on Carbon Assimilates and PEP Carboxylase Activity

Carbon metabolism in leaves of Cyamopsis exposed to e[CO_2] remained relatively higher than ambient grown plant varieties. Total Carbohydrate concentration in the leaves was higher to one to two fold in elevated exposed (Fig. 5. (b) and (c)) plants that are the cause of higher plant biomass, increased photosynthetic carbon assimilation and leaf area. Carbohydrates are the energy source of all the plant physiological process, including plant growth and respiration. Plant sugars act as a messenger in processes related to plant growth, development and play essential role in metabolic processes of physiological nature. Our results depict the enhancement in carbohydrate concentrations under elevated exposed plant with RGC 1066 showing two-fold higher concentration than RGC 1002 plant variety.

Moreover, the overall increment in sugar was contributed by sucrose, which is significantly higher in RGC 1066 plant variety and comparatively low in RGC 1002 and starch that is equally contributed by both the plant varieties. Thus, it can be inferred that under e[CO_2] concentration sucrose content increased in excess in RGC 1066 that causes an overall increment in plant sugars. The considerable differences noticed in sucrose and starch plays a central role in regulation of photosynthesis (Poorter *et al*. 2003) and the previous hypothesis confirms that sucrose cycling takes a major role in carbohydrate signaling. Moreover, the photosynthetic genes are regulated by the hexokinases that phosphorylates fructose and glucose (sucrose metabolic products), thus acting as sugar sensors. Similarly, the carbohydrate content in pods and seeds also remained comparatively higher in e[CO_2] exposed plants than ambient grown plant varieties. RGC 1066 varieties showed more accumulation of sucrose and starch in pods and seeds than RGC 1002 plant varieties thus showing better adaptation strategies under e[CO_2] concentrations indicating active photosynthesis. Active photosynthesis in the varieties may be the main cause that is regulated by the increased amount of sucrose in the leaves as the data is justifying it thus channelling sugars to pods and seeds (sink). Phosphoenolpyruvate carboxylase (Pep C) activity reported in Cyamopsis varieties remained higher in elevated concentration grown plant varieties although it does not play a central role this takes part in anaplerotic reactions replenishing TCA (Tricarboxylic acid cycle) with intermediates that take part in biosynthetic pathway and nitrogen metabolism. During pre-flowering stage Pep C activity was significantly higher under e[CO_2] in both RGC 1002 and RGC 1066 cultivars however it declined in later post- flowering stages. Previous studies suggests that it supplies malate as –COO- radical that maintains cytoplasmic pH to be constant and produces NADPH as reductive power that is required for nitrate reduction and its assimilation in nitrogen metabolism (Rawsthrone *et al*., 1980). It was well documented that this pathway leads to the formation of amino acids especially glutamine from glutamate and asparigine from aspartate by transamination in many legumes (Schramm *et al*., 1982) as oxaloacetate formed provides carbon skeleton for synthesis of asparigine from aspartate.

Effect of e[CO_2] on C/N and NPK

Total nitrogen in leaves of both the varieties declined under e[CO_2] concentrations while total organic carbon content enhanced in both the varieties resulting in enhancement of C/N ratio. C/N ratio approaches far in elevated grown plants than ambient carbon dioxide concentration grown ones thus indicating nitrogen limitation and carbon enhancement in leaves tisssue thus plant is acquiring and accumulating faster and

greater carbohydrate than nitrogen, there by causing nitrogen content to decrease but at the same time nitrogen fixation is supplementing it through bacteria like rhizhobia in nodulated roots increasing nitrogen mobilisation in leaves thus plant growth and biomass is not altered greatly .More over nitrogen content results were contradictory in seeds in comparison to leaves tissue thus no limitation in nitrogen content is noticed in seeds which results in increase in pod number and seed weight. Seeds C/N ratio was not significantly affected under e[CO_2] in RGC 1066 than RGC 1002 depicting proportional allocation of N and C to seeds. Thus e[CO_2] concentration had no negative effect to seed qualitative and quantitative characteristics in these varieties and RGC 1066 was showing better adaptive strategies under e[CO_2] concentration. Soil NPK was also found to be affected under e[CO_2] concentration. Soil nitrogen declined under e[CO_2] concentration in comparison with a[CO_2] concentration depicting that oxidised or reduced form of nitrogen is translocated faster in elevation exposed plants which results in declining of soil nitrogen in elevation exposed soil. Nitrogen transformation is chiefly attributed by bacteria but chemically or biologically transformation is poorly known. Moreover, nitrogen content in leaves of both the varieties are not significantly affected and so no change was noticed. This can simply be explained by the assimilation and utilization of translocated N salts in plant growth and enhanced biomass. On contrary soil phosphorus concentration was more in e[CO_2] exposed plants depicting either restricted translocation of phosphorus to the plant or may be the exudation of acid phosphates by enzymatic bacterial activity in form of phosphate esters in organic matter. Earlier studies provides justification of increase in total acid phosphate enzymatic activity by Gifford (Gifford *et al*. 1986; Koumeleh *et al*., 2006). Soil phosphate was found markedly increased in soil from pots that were exposed to e[CO_2] concentration on contrary to a[CO_2] concentration grown plant varieties. Leaves of both the varieties were showing a decline in the phosphorus content under e[CO_2] concentration which confirms restricted uptake and mobilisation of phosphorus from the soil. Thus e[CO_2] concentration benefits plants on the soil limited in phosphorus and increased exudation of phosphates could be an important driver of ecosystem. Previous reports suggest that under elevation decrease in soil pH by 1 unit increases the available phosphate ratio by 10 folds (Koumeleh *et al*.2006). Thus a change in pH works as driving force for increase in solubilities of available phosphates in form of salts of phosphates. Total potassium was more in e[CO_2] exposed soil than a[CO_2] exposed soil that can be correlated by decline in pH which probably promotes solubility and precipitation patterns under e[CO_2] concentration. Similarly, leaves of both the legumes varieties were showing the decline in available potassium in leaves due to restricted translocation under e[CO_2] concentration or its increased utilisation and mobilisation in plants. Seeds were balanced in nitrogen and carbon content (C/N=1) as contradictory to other C_3 crops as seeds nutritional quality is not altered under changing environment which is utmost concern nowadays for food security in agricultural country India. Thus, proper partitioning and translocation of sugars and amino acids occurred in seeds tissues during reproduction and development. Seeds nitrogen content enhanced can be simply explained by plant allocates acquired nitrogen more to the reproductive part in post flowering stages which involves nitrogen re translocation from vegetative parts. Previous experiments support that legumes exhibited more pronounced nitrogen acquisition and enhanced seeds production then non-legumes due to nitrogen-fixing symbiotic bacteria which promotes nodules formation and nitrogen assimilation on high C inputs. Total phosphorus and potassium showed neutral effects suggesting elevated carbon dioxide does not restrict translocation of these nutrients in reproductive part of the plant during tissue development.

Conclusion

It can be concluded from the above experimental study that under e[CO_2] concentration overall plant growth and biomass is enhanced due to higher photosynthesis in both the plant varieties with RGC 1066 showing double enhancement in plant biomass than RGC 1002. The cause of enhancement can be explained by higher photosynthetic rate, decreased stomatal conductance, optimal temperature and increased leaf area. Leaf area enhancement can be linked with more stomata per unit area in leaves as well as more accumulation of chlorophyll pigments in leaves tissues. Photosynthetic rate enhancement can be justified by the increased level of carbohydrates, sucrose and starch content that was the outcome of higher carbon dioxide fixation. RGC 1066 pods and seeds were found to be storing more carbohydrates, sucrose, and starch comparitively to RGC 1002 plant varieties, thus increasing source-sink capacity. Moreover, the C/N ratio was also not significantly affected in RGC 1066 under e[CO_2] enhancement than a [CO_2] concentration grown plants which is the outcome of higher carbon fixation in comparison to nitrogen assimilation and enhanced utilization that causes a dilution effect causing an effective increase in carbon metabolites. Thus, it can be concluded that RGC 1066 is better than RGC 1002 plant variety which is adapting and performing in a better way under e[CO_2] concentration.

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Abbreviations

Pep C, Phosphoenolpyruvate carboxylase;
NPK, Nitrogen, Phosphorus and Potassium;
TOC, Total organic carbon;
e[CO_2],elevated carbon dioxide;
a[CO_2], ambient carbon dioxide.

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