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Research Article

IMPACT OF CO_2 ENHANCEMENT ON PHOTOSYNTHESIS AND PROTEIN PROFILE - RESPONSE STUDIES WITH A CO_2 RESPONSIVE BLACK GRAM GENOTYPE

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ABSTRACT: Black gram (*Vigna mungo* (L.) Hepper) var. IC-282009 - a highly CO_2 responsive genotype for biomass and seed yield was grown in Open top chambers (OTCs) under three levels of CO_2 i.e. ambient (390 ppm) and two elevated levels 550ppm and 700ppm to assess photosynthetic acclimation to elevated CO_2. Net photosynthetic rate (P_N), change in leaf soluble protein profile and leaf carbohydrate constituents such as total soluble sugars, reducing sugars and starch content in leaves was quantified at all three CO_2 concentrations. Photosynthetic rate was enhanced by 78% and 30% at flowering stage with 550ppm and 700ppm CO_2 as compared with ambient control. It was also observed a higher accumulation of starch, total soluble sugars and reducing sugars in leaves at elevated CO_2 levels. However, the leaf protein content recorded a decrease and altered the profile of ploy peptides with enhanced CO_2 levels. At elevated CO_2 concentrations significant differences were observed in ploy peptide profile at vegetative and flowering stages, the intensity of 260 kDa poly peptide increased at vegetative stage, whereas 72 kDa polypeptide increased at flowering stage, while 52 kDa poly peptide decreased at both stages. Enhanced CO_2 concentrations improved the P_N though certain polypeptides of leaf protein are down regulated and necessitate further experimentation to confirm their involvement in responsiveness of the selected black gram genotype.

Key words: Black gram, CO_2 responsive genotype, Elevated CO_2, OTCs, Protein profile.

INTRODUCTION

The present global atmospheric CO_2 concentration is expected to rise 500-1000 ppm by the year 2100 (IPCC, 2007). The present atmospheric CO_2 is an important limiting factor for the photosynthesis, growth and productivity of many crop species. Increased atmospheric CO_2 concentration is known to directly influence photosynthesis as a result of being its substrate. For the past several decades there have been numerous reports about the response and acclimation of plant photosynthesis to enhanced atmospheric CO_2 concentration. CO_2 enrichment generally increases net photosynthesis in C_3 species and reduces photorespiration (Long *et al*. (2006), increases biomass, seed yield and improves water use efficiency. However for some plant species with longer exposure to elevated CO_2 resulted in acclimation of photosynthesis with down-regulation of the amount of Rubisco protein (Rowland *et al*. 1991), though other species showed minimal down regulation. The response for maximum photosynthetic capacity at elevated CO_2 condition will be influenced by many factors such as species, age, strength of source and sink, as well as abiotic factors like moisture availability, nutrition status and temperature.

Increased carboxylation at elevated CO_2 affects leaf components, at least in part as a response to increased carbohydrate production and metabolism. The coarse control of Rubisco protein probably serves to optimize CO_2 acquisition with utilization of the fixed carbon. Leaf carbohydrate concentration has long been associated with maintaining the balance between assimilate production and its consumption by sinks, and carbohydrates are known to modulate the expression of many photosynthetic and non photosynthetic genes.

The present study aimed to assess the photosynthetic acclimation and related parameters of CO_2 responsive black gram crop plant.

MATERIALS AND METHODS

The blackgram genotypes with different yield potentials were assessed for their response to elevated CO_2 (550 & 700ppm) for biomass and seed yield and var. IC-282009 was reported as the most responsive genotype (CRIDA, 2011).

Plant material and growth conditions

Six Open Top Chambers were used to raise black gram (*Vigna mungo* L.) var. IC-282009 plants from sowing to harvest under three levels of CO_2 i.e. ambient (390ppm), two elevated (550ppm and 700ppm) concentrations. The open top chambers (OTCs) are with 3m x 3m x 3m dimensions and lined with transparent PVC (polyvinyl chloride) sheet having 90% transmittance of light. The elevated CO_2 of 550ppm and 700ppm were maintained in two OTCs each and two OTCs without any additional CO_2 supply served as ambient control. The CO_2 concentrations within the OTCs were maintained and monitored continuously throughout the experimental period as illustrated by Vanaja *et al*. (2006). One plant for pot of 5 lts capacity was maintained in five replications for each concentration of CO_2. Seeds were inoculated with *Rhizobium* before sowing and plants were maintained stress free by providing regular irrigation. The observations were recorded on three replications at vegetative (26 DAS), flowering (35 DAS) and pod setting (42 DAS) growth stages.

Photosynthetic measurements

Net photosynthetic rate (P_N) of fully expanded young leaves was measured at vegetative and flowering stages with a portable photosynthesis system (LI-6400, LI-COR). Photosynthetic measurements were performed between 10:00 and 12:00hrs, with irradiance was set at 1200 µmol m^{-2} s^{-1} and by inserting CO_2 cylinder similar elevated CO_2 levels in the leaf chamber were maintained.

Leaf samples were drawn between 10:00 and 12:00hrs for analysis of leaf soluble proteins, total soluble and reducing sugars and starch content at three different growth stages.

Sugars and Starch

Leaf samples (0.2 g fresh wt.) for determination of sugars and starch contents were plunged into ethanol (95%) and preserved for further analysis. The estimation of reducing sugars was determined by di-nitro salicylic acid reagent method (Hedge *et al*. 1962) and the color developed was read at 540 nm using UV- visible spectrophotometer (Genesys 6, USA). The total soluble sugars and starch content was determined by phenol- sulfuric acid method (Dubois *et al*. 1956) and the dark green color formed was read at 490 nm. Contents of reducing, total soluble sugars and starch was expressed as mg g^{-1} FW.

Protein extraction and quantification

Leaves from plants grown at 3 levels of CO_2 were sampled at each growth stage were immediately frozen in liquid nitrogen. The leaf total soluble proteins were extracted as per Guy *et al.* (1992) with slight modification. This consisted of homogenization with a chilled mortar and pestle using a buffer containing ice-cold 50 mM Tris-HCl (pH.7.5), 2 mM EDTA and 0.04% (v/v) 2-mercaptoethanol (Sigma). The homogenate was centrifuged at 4000 rpm for 30 minutes at room temperature and supernatant was re-centrifuged for 20 minutes and stored at -20°C for further analysis (Hames and Rickwood 1990).

Protein extracts were thawed and their concentration determined by Bradford *et al*. (1976) method with Coomassie Brilliant Blue G-250. The extract was added to Bradford reagent and the intensity of colour read spectro-photometrically at 595 nm. The standard graph was plotted by using BSA (Sigma) and the quantity of protein was expressed as mg g^{-1} FW.

Protein Electrophoresis (SDS-PAGE)

100 µg of the leaf protein was mixed with 1:1 gel loading buffer containing 62.5 mM Tris-HCl (pH 6.8), 2% SDS, 5% (v/v) 2-mercaptoethanol, 10% glycerol and 0.01% bromophenol blue and then heated at 100°C for 2 minutes and given a short spin for homogenation. SDS PAGE (Laemmli *et al*. 1970) was performed by loading 100 µg of the extracted protein samples into wells of the 12% of the SDS polyacrylamide gel along with standard protein ladder (Fermentas Spectra multicolor broad range of protein 260 to 10 µg). The gels were fixed in trichloro acetic acid and stained with solution containing 0.1% (w /v) Coomassie brilliant blue G-250, 10% (v/v) glacial acetic acid and 40% (v/v) methanol (Sigma) and destained with solution of 40% methanol, 10% glacial acetic acid. The protein banding pattern was observed under Tran's illuminator (Biovision 1000/20M, EU). The molecular weight of proteins was determined by comparing them with standard protein ladder.

RESULTS AND DISCUSSION

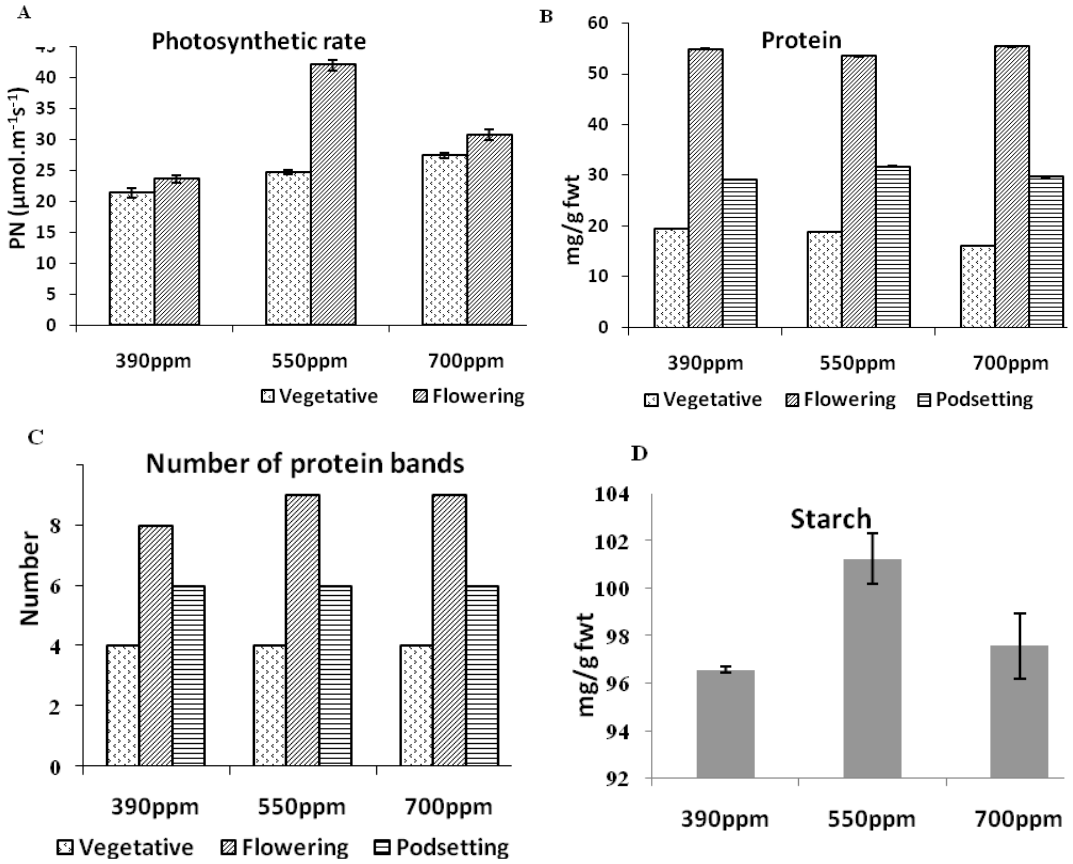
Elevated CO_2 concentration significantly affected the physiological and biochemical aspects related to photosynthesis of selected black gram genotype (Table 1). Elevated CO_2 concentrations significantly enhanced P_N at both vegetative and flowering stages and leaf total soluble and reducing sugars as well as starch contents increased with enhanced CO_2.

Decreased leaf soluble protein content and changed protein profile was observed with elevated CO_2 condition. Photosynthetic rate (P_N) was higher at elevated CO_2 than ambient control condition during vegetative and flowering growth stages (Fig.1A). An increase of 16% and 28% of P_N was recorded at vegetative stage and 78% and 30% at flowering stage with 550ppm and 700ppm CO_2 respectively over ambient control. Among the two elevated CO_2 levels, higher P_N was recorded during vegetative stage at 700ppm while, flowering stage at 550ppm. Rogers *et al*. (2009) observed that legumes show greater enhancement of photosynthesis and growth by elevated CO_2.

Table 1: Analysis of variance for physiological and biochemical parameters of black gram genotype- IC-282009

		Mean sum of squares		
Source of variation	**df**	**Photosynthetic rate**	**Leaf soluble Protein**	
Replications	2	2.127	0.017	
CO_2 levels	2	177.675**	3.12**	
Stages	1	259.9**	5966**	
CO_2 x Stages	2	106.6**	8.93**	
Error	10	0.672	0.058	
Source of variation	**df**	**Total soluble sugars**	**Starch**	**Reducing sugars**
Replications	2	0.232	4.37	0.096
CO_2 levels	2	54.24**	18.28**	0.627*
Error	4	0.046	0.83	0.077

*Significant at $p<0.05$ and ** Significant at $p<0.01$



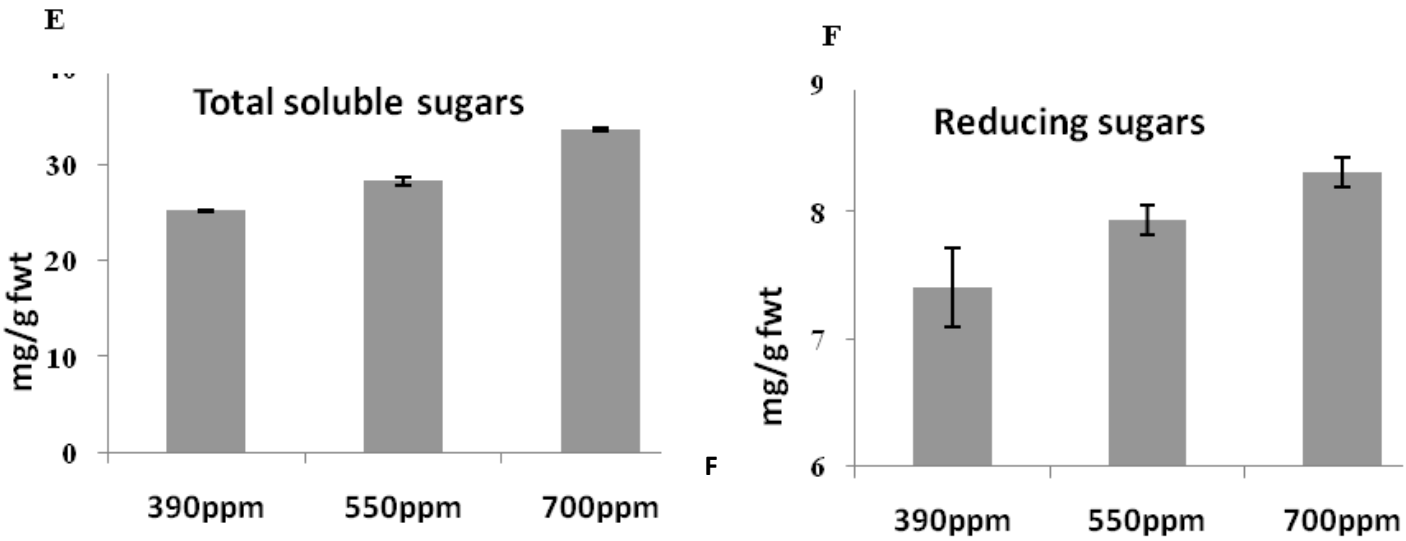


Figure. 1. A.Photosynthetic rate, B. Protein content, C. Number of protein bands at vegetative, flowering and pod setting stage; D. Starch, E. Total soluble sugars, F. Reducing sugars at flowering stage of black gram grown at ambient (390ppm) and elevated CO_2 (550ppm,700ppm)

Table 2: Rate of flow, Molecular weight (kDa) and number of protein bands of ambient and elevated CO_2 grown black gram (cv. IC-282009) plants at different growth stages

Growth stages	CO_2 Concentration (ppm)					
	390		550		700	
Band No	**Rf**	**M.wt.**	**Rf**	**M.wt.**	**Rf**	**M.wt.**
Vegetative						
1	1.58	258.4	1.62	257.2	1.63	257.4
2	3.84	81.0	3.82	82.0	3.82	82.0
3	4.20	69.0	4.16	70.1	4.16	70.0
4	5.29	*52.4*	5.20	*53.5*	5.18	*53.9*
Flowering						
1	1.95	202.6	1.72	252.9	1.76	254.9
2	3.35	76.8	3.22	85.1	3.23	85.9
3	3.65	67.7	3.47	71.7	3.53	70.8
4	3.94	51.4	3.85	55.4	3.83	55.3
5	4.73	47.6	4.59	49.3	4.61	49.6
6	5.27	36.4	5.18	37.9	5.14	38.8
7	6.42	28.8	6.32	29.6	6.28	29.7
8	7.38	27.5	7.21	28.2	7.13	28.5
Pod setting						
1	1.84	208.6	1.67	218.6	1.60	222.0
2	3.28	75.8	3.23	76.9	3.17	77.6
3	3.54	63.2	3.51	64.6	3.52	65.1
4	3.97	50.5	3.95	50.9	3.86	51.2
5	4.75	47.2	4.70	47.2	4.69	47.5
6	5.27	35.2	5.25	36.0	5.22	36.9

At the vegetative stage, there were 4 clear poly peptides (Fig.1C) at all the three CO_2 levels with molecular weights of 258.4, 81.0, 69.0 and 52.4 kDa (Table.2). It is interesting to observe that with increased CO_2 concentration, the intensity of 52 kDa poly peptide registered a decrease at both 550ppm and 700ppm, whereas, a significant increase in 257 kDa polypeptide as compared with ambient protein profile (Fig.2A).

At the flowering stage there were eight polypeptides at three CO_2 levels which include the four poly peptides at vegetative stage along with four new poly peptides of 47.6, 36.4, 28.8, 27.5 kDa (Table.2). One new polypeptide band was appeared only at elevated CO_2 levels of 550ppm (26.4 kDa) and 700ppm (26.6 kDa). Among these polypeptide bands, elevated CO_2 conditions significantly decreased 51.4 kDa polypeptide and increased 70.8 kDa polypeptide (Fig.2B). At pod setting stage the differences are minimal at all the three CO_2 levels and protein profile was similar with six clear poly peptides (Fig.2C).

Accumulation of starch (Fig.1D) total soluble sugars (Fig.1E), reducing sugars (Fig.1F) were significantly higher at elevated CO_2 conditions than the ambient control during flowering stage. The linear increase in the content of total soluble sugars and reducing sugars was recorded with increase in CO_2 concentration from 390ppm to 700ppm. Meta analysis of response crops to elevated CO_2 revealed that sugars and starch contents were increased by 30-40% per unit leaf area due to increased photosynthetic activity (Ainsworth and Long 2005; Ainsworth 2008). The selected black gram (*Vigna mungo* L.) var. IC-282009, showed a linear increase in photosynthetic rate regardless of decrease in leaf soluble protein content at elevated CO_2 levels as compared with ambient condition. The protein content and profile varied with different growth stages of the crop in response to different levels of CO_2. Enhanced CO_2 concentration decreased the intensity of 52 kDa and 51.4 kDa polypeptide at vegetative and flowering stages. Several investigations suggest that most prominent change in leaf photosynthetic apparatus under elevated CO_2 is a decrease in the amount of Rubisco protein (Drake *et al*., 1997). Pandurangam *et al*. (2006) reported the photosynthetic acclimation to elevated CO_2 concentration due to down regulation of Rubisco through limitation imposed on Rubisco small subunit gene expression, as a consequence of sugar accumulation in wheat leaves. However, in sunflower and mung bean they reported no down regulation of photosynthetic rate under elevated CO_2 condition.

From the above results it can be concluded that under elevated CO_2 condition higher P_N were recorded though the leaves accumulated excess starch in selected CO_2 responsive black gram genotype. Furthermore, elevated CO_2 significantly affected leaf soluble protein content and the banding pattern of polypeptides (52 kDa). The enhanced photosynthetic rate even at down regulation of certain polypeptides (52 kDa) needs further quantification of these polypeptides role in CO_2 assimilation and to ascertain their role in responsive of the present genotype to elevated CO_2.

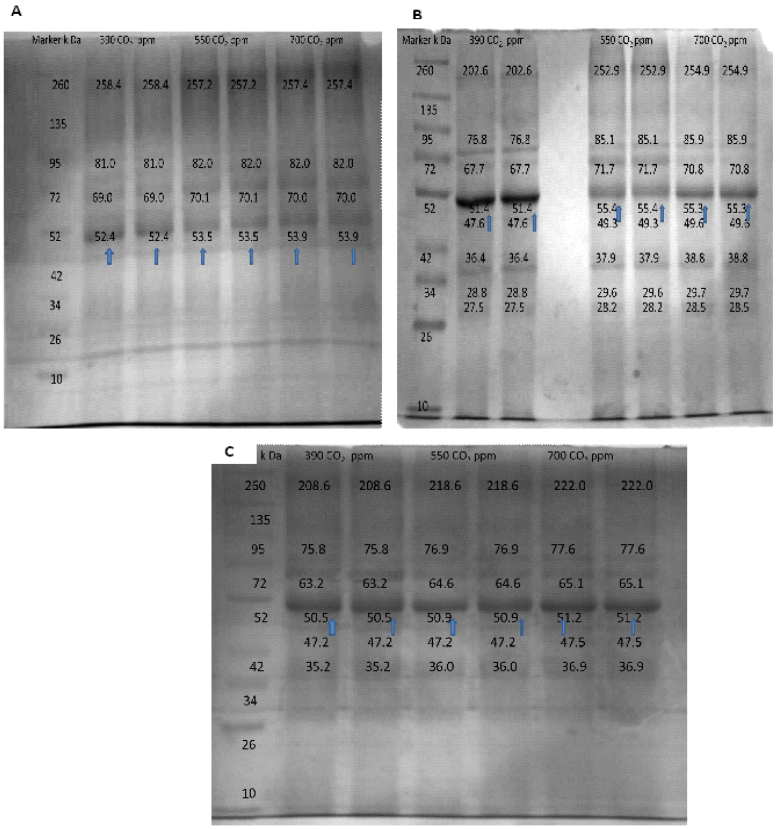


Figure. 2. Leaf soluble protein SDS-PAGE of blackgram var. IC-282009 of ambient (390ppm) and elevated CO_2 level (550ppm, 700ppm), A. At vegetative, B. flowering, C. Pod setting

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