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Carbohydrates and Sucrose Metabolizing Enzymes in the Leaves of *Vigna mungo* Genotypes as Influenced by Elevated CO_2 Concentration

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ABSTRACT

Effect of different CO_2 concentrations on sucrose metabolizing enzymes and on carbohydrate metabolism was studied for eight blackgram (*Vigna mungo* L. Hepper) genotypes grown in open top chambers under ambient (380 µmol mol^{-1}) *vs.* elevated CO_2 (550 and 700 µmol mol^{-1}) levels. The higher acid invertase activity over neutral invertase indicated the major role of acid invertase in sucrose breakdown. Higher acid invertase activity over Sucrose Synthase (SuSy) suggested the major role of invertase in sucrose breakdown and sucrolysis. Sucrose Phosphate Synthase (SPS) activity did not match with sucrose pool sizes in mature leaves and rather varied among genotypes. Plants exposed to higher CO_2 concentrations showed higher starch and sucrose contents as compared with those exposed to ambient CO_2. Leaf starch content being found several-folds higher than sucrose throughout the study indicated its major role in regulating assimilate partitioning. Increase in glucose *vs.* fructose concentrations for genotypes grown under elevated CO_2 conditions ranged from 20 to 90% and from 10 to 140%, respectively. The hexoses/sucrose ratio for elevated CO_2 concentration was approximately 0.8-1.6, however for ambient CO_2 content it approximately amounted to unity. Genotypes IC436720, IC519805, IC343952, and IC282009 with low hexose/sucrose ratio representing high CO_2 assimilation along with high sucrose formation indicated better tolerance to elevated CO_2 for carbon partitioning and carbohydrate metabolism. The up-regulation of leaf carbohydrate metabolizing enzymes of low hexose/sucrose as well as low sucrose/starch ratios for the genotype IC436720 (as compared with other genotypes) improved its photosynthetic capability which coupled with its better efficiency of carbon partitioning (indicative of better acclimation to elevated CO_2) could prove beneficial to its growth and productivity in the future change of climatic conditions.

Keywords: Enzymes, Invertase, Soluble sugars, Sucrose metabolism, Up-regulation.

INTRODUCTION

The current atmospheric CO_2 concentration (380 µmol mol^{-1}) limits the photosynthetic potential, growth and productivity of many agricultural crops and exerts different effects on the plant, depending upon the species and its developmental stage. Among these, C_3 species including legumes show great potential to rising CO_2 (Drake *et al*., 1997). Under elevated CO_2 conditions, legumes are able to shunt excess carbon to root nodules where it can serve as a carbon and energy source for the bacterial symbionts. In effect, legumes exchange the excess carbon for nitrogen and thereby maximize the benefits of elevated atmospheric CO_2. Compared to other plant species, legumes show greater enhancement of photosynthesis and growth by the elevated CO_2 (Rogers and Ainsworth, 2009). *Vigna mungo* a short duration legume crop grown in India shows increased

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productivity at elevated CO_2 levels (Vanaja *et al.,* 2006). A higher concentration of CO_2 is likely to profoundly affect the growth, physiology and biochemistry of plants (Ziska, 2008).

Elevated CO_2 also leads to an increase in the global atmospheric level of warming. These changes in CO_2 and temperature not only influence climate but also the crop productivity by affecting plant photosynthetic efficiency, carbohydrate metabolism and related enzyme activities for major crops. Plants could mitigate these changes through conversion of atmospheric CO_2 into carbohydrates and other beneficial organic compounds. These carbohydrates get accumulated in plants under elevated CO_2 due to increased photosynthetic rates (Ainsworth and Rogers, 2007). Increase in photosynthesis is reflected in the harvestable yield of crops (wheat, rice and soybean) under elevated CO_2 concentration (Long *et al.,* 2006). The key enzymes involved in carbon utilization process are Sucrose-Phosphate Synthase (SPS), Sucrose Synthase (SuSy, a glycosyl transferase) as well as invertase. Elevated CO_2 has been reported to increase the activity of SPS in rice (Hussain *et al.,* 1999) and soybean (Vu *et al.,* 2001) compared to ordinary ambient CO_2 conditions. The accumulation of the primary photosynthetic products, i.e. sucrose and starch, as well as the activities of the key enzymes responsible for their metabolism, are controlled and regulated through atmospheric CO_2 levels. Photosynthetic acclimation to elevated CO_2 is often attributed to carbohydrate feedback effects, which are thought to be linked to the sensing of increased soluble sugars resulting from carbohydrate sink saturation. However, the degree of feedback inhibition of photosynthesis and the form in which excess carbohydrate is stored may differ considerably among species (Bowes, 1993). With the increase of CO_2 concentration, rate of photosynthesis increases, and this ultimately increases the sucrose synthesis and the total leaf sucrose level. The hydrolytic decomposition of sucrose into fructose and glucose increase cell sugar concentration. The generated hexose molecules get phosphorylated by hexokinase, which act as sugar sensor molecules and initiates a signal cascade (via sucrose cycling) that results in the repression of a number of photosynthetic genes (Vara Prasad *et al*., 2009). The capacity of starch synthesis under high CO_2 enables plant to achieve a high rate of photosynthesis by utilization of triose-phosphate molecules (Paul and Foyer, 2001).

The present investigation is an attempt to understand the pattern of activities of sucrose metabolizing enzymes as well as carbohydrate content for blackgram (*Vigna mungo* L. Hepper) genotypes raised under ambient *vs.* elevated CO_2 concentrations.

MATERIALS AND METHODS

Eight blackgram (*Vigna mungo* L. Hepper) genotypes IC587753, IC436720, IC519805, IC282009, IC343952, IC436610, IC281987 (representing indigenous collection from Andhra Pradesh, India) and T-9 (National Check) were sown in pots in October, 2011 in six Open Top Chambers (OTCs) having the dimensions of 3×3×3 m, covered with transparent PVC (Polyvinylchloride) sheets of 90% light transmittance. Growing conditions represented ambient (380 μmol mol^{-1}) and elevated (550 and 700 μmol mol^{-1}) CO_2 concentrations maintained in two OTCs each. The elevated levels of CO_2 into these chambers were maintained by continuously injecting 100% CO_2 into plenum, while two OTCs were maintained at ambient CO_2 level (380 μmol mol^{-1}) serving as control chambers. The CO_2 concentration was maintained with the help of solenoid valves, rotameters, PCs, Program Logic Control (PLC) and Supervisory Control as well as Data Acquisition (SCADA) software. After every 3 minute intervals air sample was taken from each chamber and analyzed through Non-Dispersive Infrared (NDIR) CO_2 analyzer (California Analytical). Leaf samples were taken for enzyme and metabolite extraction during early pod filling stage of the crop.

Enzyme Extraction and Sucrose Phosphate Synthase (SPS) Assay

Leaf material (1.0 gm) was homogenized into 4.0 ml of 50 mM MOPS-NaOH buffer (pH 7.5) containing 15 mM $MgCl_2$, 1 mM EDTA, 2.5 mM DTT and 0.1% (v/v) Triton X-100. The extract was centrifuged at 8000 rpm for 10 minutes at 4°C, the clear supernatant being taken for enzyme assay. The reaction mixture contained MOPS-NaOH buffer 100mM (pH 7.5); NaF 1 mM; $MgCl_2$ 12 mM; UDPG 8 mM; fructose 6-phosphate 8 mM and enzyme 200 µl. Blank, containing heat denatured enzyme was run in parallel with the reaction mixture. Reaction mixture was incubated at 37ºC for 20 minutes. Following incubation, reaction was terminated by the addition of equal volumes of 30% KOH. All the unreacted fructose 6-phosphate was eliminated by keeping the tubes in boiling water bath for 10 minutes. Released sucrose-6 phosphate was determined through resorcinol-thiourea method. An appropriate volume of resorcinol-thiourea and HCl: water (5:1) was added to the reaction mixture and kept at 80ºC for 8 minutes for colour development. Following incubation, the reaction mixture was cooled and the absorption read at 520 nm. SPS activity was expressed as nmole mg^{-1} protein min^{-1}.

Sucrose Synthase (SuSy) Assay

Reaction mixture contained MOPS-NaOH 50 mM (pH 7.5); $MgSO_4$ 15 mM; UDPG 25 mM; fructose 25 mM and the enzyme 100 µl. Blank contained heat denatured enzyme in addition to all the components in the reaction mixture. Following incubation of reaction mixture at 37ºC for 20 minutes the reaction was terminated by an addition of an equal volume of 30% KOH. Further the reaction mixture was boiled for 10 minutes to eliminate all the unreacted fructose. The release of sucrose was determined through resorcinol-thiourea method as described earlier for SPS. The SuSy activity was expressed as nmole mg^{-1} protein min^{-1}.

Invertase Assay

Soluble invertase was assayed at 37°C in a reaction mixture containing 100mM buffer (citrate phosphate pH 5.0 for acid invertase and MOPS-NaOH buffer pH 7.0 for neutral invertase) and 50 mM of sucrose (Huber, 1989). The reaction was initiated by adding 100 µl of crude enzyme followed by incubation at 37°C for 20 minutes. Reaction was terminated by keeping the samples in boiling water bath for 10 minutes. The produced glucose plus fructose were determined through DNS method (Miller, 1972). Invertase activity was expressed as nmole mg^{-1} protein min^{-1}.

Soluble Sugars Content

Leaf samples (250 mg) were homogenized with 5 ml of 80% ethanol and centrifuged at 8,000 rpm for 10 minutes. Supernatant was used for estimation of total soluble sugars (sucrose, fructose and glucose) as well as reducing sugars, while the pellet being used for starch estimation. Total soluble sugars were determined using Anthrone method (Dubois *et al*., 1956) while reducing sugars estimated through DNS method (Miller, 1972) and expressed as mg g^{-1} FW. Total fructose was estimated through an addition of 0.5 ml Seliwanoff reagent (0.1 g Resorcinol+0.25 g Thiourea dissolved in 100 ml glacial acetic acid) into 1.0 ml extract (water+extract). Further, 3.5 ml of HCl:water (5:1) was added and the samples kept in water bath at 80°C for 8 minutes. Following incubation, samples were cooled and observations made at 520 nm. Glucose content was found out by determination of the concentration of fructose and total reducing sugars.

For starch analysis, pellet was washed with 80% ethanol till the washings stopped giving colour against Anthrone reagent. Water and perchloric acid (52%) in the ratio of 1:1 was added to the dried pellet and centrifuged. The process was repeated twice and the supernatant so obtained used for measurement of starch content. Starch content was determined by Anthrone method (Scott and Melvin, 1956). An appropriate volume of anthrone reagent was added to diluted sample and kept at boiling for 8 minutes. After being boiled, sample was rapidly cooled and the intensity of green to dark green colour measured at 630 nm. Starch content was expressed as mg g^{-1} FW.

Total Soluble Protein

Protein concentration in the enzyme extracts was determined according to the method of Lowry *et al.* (1951), using Bovine Serum Albumin as the standard.

Statistical Analysis

The experimental data were statistically analyzed using two-way Analysis of Variance (ANOVA).

RESULTS

Invertase Activity

The overall patterns of acid and neutral invertase activities for ambient and elevated CO_2 were approximately similar for all the eight genotypes. The effect of elevated CO_2 concentration on both acid and neutral invertase activity showed significant ($P< 0.01$) differences among the genotypes. With an increase in CO_2 concentration, a decline in leaf invertase activity (both acid and neutral invertase) was observed for IC587753, IC436720, IC519805 and IC343952, while, for the other set, namely: IC282009, IC436610, IC281987 and T-9, it increased. Overall, the activity of acid invertase was observed to be higher than that of neutral invertase. At an elevated CO_2 level (700 µmol mol^{-1}), the decline in neutral invertase activity was observed by 39, 19, 53, and 43% for IC587753, IC436720, IC519805 and IC343952, respectively. However, enhancement of activity was observed for IC282009 (32% at 700 µmol mol^{-1}), IC436610 (23% at 700 µmol mol^{-1}), IC281987 (20% at 550 µmol mol^{-1}) and T-9 (72% at 550 µmol mol^{-1}) (Figure 1). Comparatively, acid invertase activity dropped by 33% (IC587753), 14%

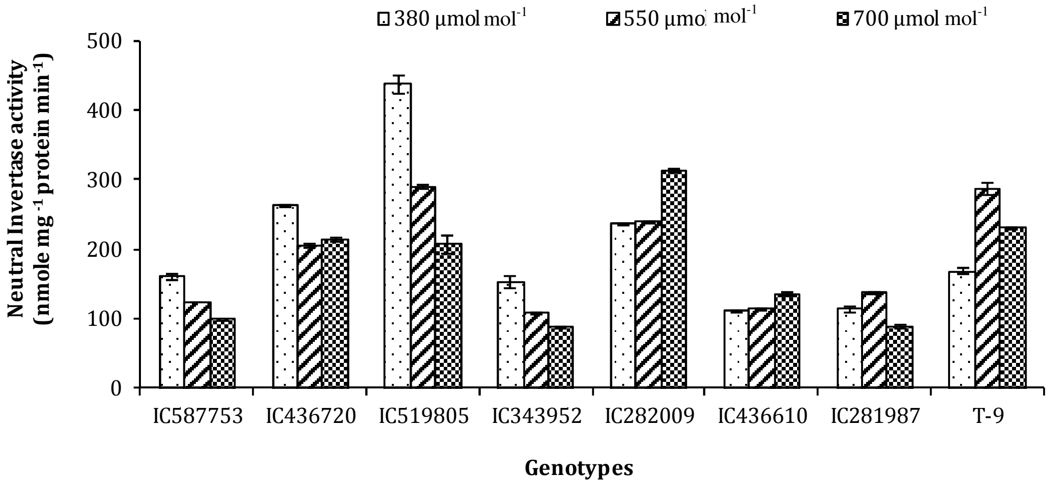

Figure 1. Neutral invertase activity for *Vigna mungo* genotypes at different CO_2 concentrations.

(IC436720), 41% (IC519805) and 45% (IC343952) at 700 µmol mol^{-1}. Conversely, increase in activity was observed by 24% (IC282009, 700 µmol mol^{-1}), 18% (IC436610, 700 µmol mol^{-1}), 28% (IC281987, 550 µmol mol^{-1}) and 15% (T-9, 700 µmol mol^{-1}) (Figure 2).

Sucrose Synthase (SuSy) Activity

Significant variability ($P< 0.01$) in SuSy activity was observed among genotypes under elevated CO_2 concentrations (Figure 3). Genotypes demonstrated a differential pattern of SuSy activity at different CO_2 concentrations. Optimum CO_2 concentration for SuSy activity was 380 µmol mol^{-1} (IC436720, IC519805, IC281987), 550 µmol mol^{-1} (IC587753, IC282009) and 700 µmol mol^{-1} (IC343952, IC436610, T-9). Maximum change in SuSy activity at CO_2 elevated level (700 µmol mol^{-1}) was observed for IC436720 (a decrease by 44%) and T-9 (an increase by 15%). An interesting observation as regards SuSy activity was noticed for IC343952, which showed drastic decrease in activity at 550 µmol mol^{-1} while a further rise of CO_2 concentration to 700 µmol mol^{-1} led to a revival of SuSy activity.

Sucrose Phosphate Synthase (SPS) Activity

Genotypic variation in SPS activity was observed with CO_2 concentrations. Elevated CO_2 led to significant ($P< 0.01$) levels of SPS activity as compared with the control conditions. SPS activity increased linearly with CO_2 concentrations for IC436720, IC282009, IC436610 and T-9 while a reverse trend was observed for IC281987. Elevated CO_2 to 700 µmol mol^{-1} led to increases in SPS activity by approximately 302, 178, 396 and 310% for IC436720, IC282009, IC436610 and T-9, respectively while the activity decreased by 75% for IC281987. A maximum increase in SPS activity under elevated CO_2 (700 µmol mol^{-1}) was observed for IC436610 (396%). Genotype IC587753, IC519805 and IC343952 revealed variable responses to CO_2 concentrations. A decrease in SPS activity was observed for IC587753 and IC343952 at 550 µmol mol^{-1} CO_2 concentration, followed by a slight revival trend at 700 µmol mol^{-1}. With respect to 380 µmol mol^{-1}, the activity decreased by 44% (IC587753), 62% (IC343952) at 550 µmol mol^{-1} and by 40% (IC587753) and 41% (IC343952) at 700 µmol mol^{-1}. A sharp increase (46%) in SPS activity was observed in IC519805 at 550 µmol mol^{-1}, but with

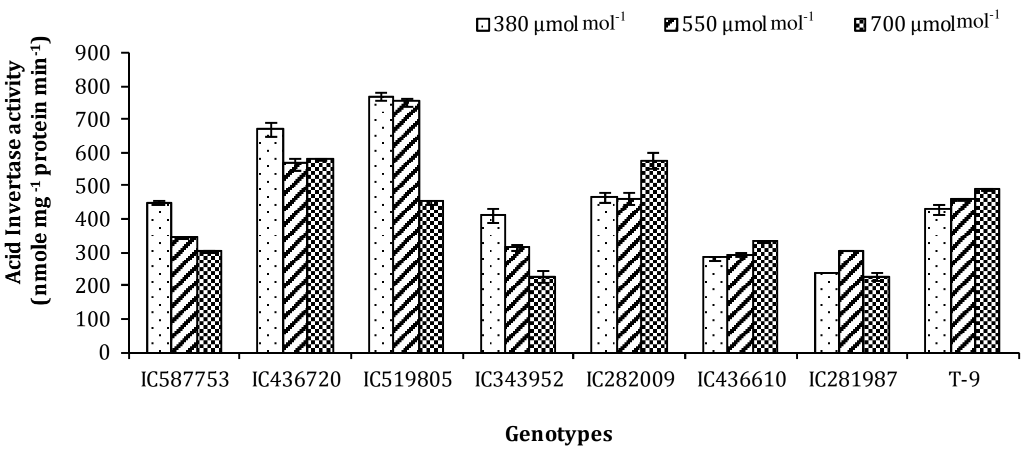

Figure 2. Acid invertase activity for *Vigna mungo* genotypes at different CO_2 concentrations.

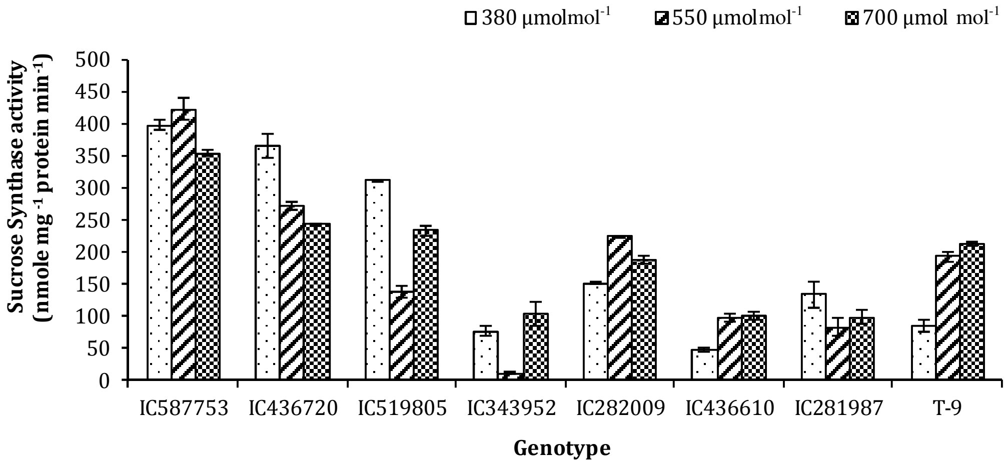

Figure 3. Sucrose synthase activity for *Vigna mungo* genotypes at different CO_2 concentrations.

further increase of CO_2 concentration (700 µmol mol^{-1}) it was observed to decrease (Figure 4).

Carbohydrate Metabolism

The ANNOVA results for different parameters of CO_2 concentration, genotypes and interaction of genotypes *vs.* CO_2 concentration were highly significant for fructose and starch, however glucose and sucrose showed significant level only for genotypes. Similarly, significant response at CO_2 concentration, genotypes and interaction of CO_2 with genotypes was also observed for hexose/ sucrose and sucrose/starch ratio. The fructose ($P< 0.01$) and glucose ($P< 0.01$) concentrations increased significantly in CO_2 enriched conditions (550 and 700 µmol mol^{-1}) as compared to the ambient (380 µmol mol^{-1}) for all the genotypes. Glucose concentration was variable among genotypes in elevated CO_2 conditions. Glucose concentrations were 20-90% higher at 700 µmol mol^{-1} for IC436720, IC343952, IC436610, IC281987 and at 550 µmol mol^{-1} for IC519805, and T-

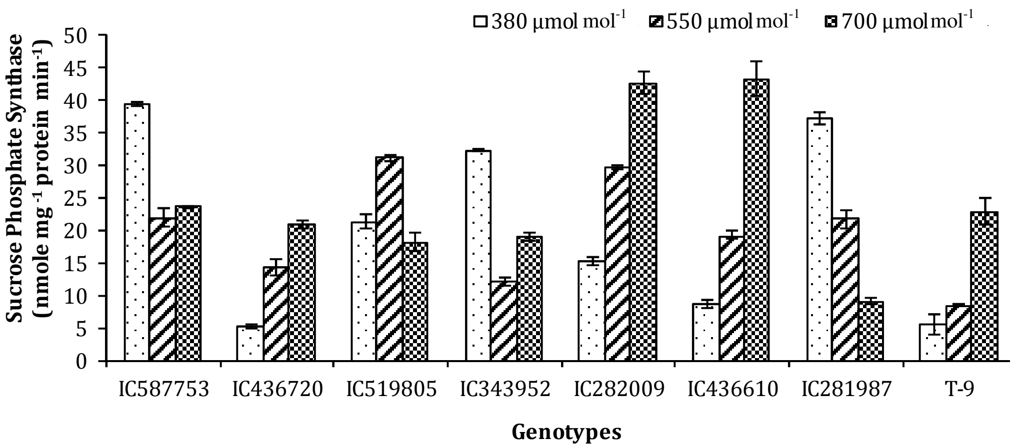

Figure 4. Sucrose phosphate synthase activity for *Vigna mungo* genotypes at different CO_2 concentrations.

9. In contrast, increasing CO_2 concentration reduced glucose levels in IC587753 (3%) and IC282009 (36%). The highest increase in glucose level was observed for IC343952 by 89%. In agreement with results of glucose, mean fructose levels also differed among genotypes with CO_2 concentrations. Compared with glucose level, fructose level always increased at elevated conditions for all the genotypes. Enhancement in fructose level ranged from 9 to 143% among genotypes at CO_2 enriched conditions (Table 1). The CO_2 enriched genotypes had significantly (P< 0.01) higher percentages of sucrose and starch compared with ambient CO_2 levels in all the genotypes studied. The enhanced CO_2 levels led to decline in sucrose and starch contents in IC282009. Contrary to IC282009, other genotypes showed higher accumulation of sucrose and starch at elevated CO_2 concentrations, although the level of these osmolytes had remained significantly low under low CO_2 conditions (Table 2).

The hexose/sucrose concentration was significant (P< 0.01) at elevated CO_2. Low hexose/sucrose ratio was observed in IC436720, IC436610, IC343952 and IC282009 at ambient CO_2 conditions. Elevated CO_2 levels raised hexose/sucrose

Table 1. Hexose concentrations for *Vigna mungo* genotypes at different CO_2 concentrations.

Genotypes	Glucose (mg g^{-1} FW)			Fructose (mg g^{-1} FW)		
	CO_2 concentration (μmol mol^{-1})					
	380	550	700	380	550	700
IC587753	2.70 ± 0.46	2.34 ± 0.49	2.62 ± 0.52	11.65 ± 0.75	16.88 ± 0.93	16.25 ± 0.07
IC436720	1.88 ± 0.49	1.29 ± 0.09	2.77 ± 0.61	9.19 ± 0.28	11.74 ± 0.04	17.83 ± 0.04
IC519805	1.80 ± 0.52	3.24 ± 1.42	2.20 ± 0.04	11.36 ± 0.19	23.13 ± 0.06	8.64± 0.07
IC343952	1.62 ± 0.16	2.26 ± 0.33	3.06 ± 0.49	8.93 ± 0.03	11.16 ± 0.13	16.36 ± 0.20
IC282009	5.31 ± 0.69	5.02 ± 0.12	3.42 ± 0.46	9.43 ± 0.05	10.28 ± 0.06	10.28 ± 0.08
IC436610	3.67 ± 1.13	3.36 ± 0.41	5.67 ± 3.41	12.98 ± 0.05	10.27 ± 0.09	31.51 ± 2.34
IC281987	6.38 ± 0.50	7.27 ± 6.38	9.78 ± 0.74	13.37 ± 0.09	15.54 ± 1.32	16.15 ± 1.22
T-9	3.45 ± 0.49	4.91 ± 0.75	4.59 ± 0.41	11.62 ± 0.05	15.50 ± 0.01	12.70 ± 0.02
	F value	CD (0.05)	CD (0.01)	*F* value	CD (0.05)	CD (0.01)
CO_2 conc.	*2.04*	*NS*	*NS*	*222.59***	*0.032*	*0.043*
Genotypes	*13.57***	*1.49*	*1.99*	*72.30***	*0.053*	*0.070*
CO_2 conc. ×Genotypes	*1.09*	*NS*	*NS*	*109.88***	*0.091*	*0.122*

** Significant at *P*< 0.01.

Table 2. Sucrose and starch concentrations for *Vigna mungo* genotypes at different CO_2 concentrations.

Genotypes	Sucrose (mg g^{-1} FW)			Starch (mg g^{-1} FW)		
	CO_2 concentration (μmol mol^{-1})					
	380	550	700	380	550	700
IC587753	8.22 ± 0.32	8.19 ± 0.41	9.35 ± 0.36	28.5 ± 0.67	40.93 ± 1.77	33.98 ± 3.68
IC436720	6.95 ± 0.26	7.36 ± 0.02	10.8 ± 0.99	57.45 ± 1.63	85.72 ± 2.03	117.2 ± 1.38
IC519805	6.6 ± 0.30	12.5 ± 1.05	7.13 ± 0.10	46.3 ± 0.79	102 ± 4.51	64.37 ± 3.84
IC343952	6.67 ± 0.06	8.11 ± 0.04	10.6 ± 0.40	58.6 ± 1.63	90.02 ± 2.82	117.9 ± 2.93
IC282009	10.1 ± 0.43	10 ± 0.10	8.66 ± 0.24	57.5 ± 0.65	35.53 ± 0.67	15.66 ± 0.18
IC436610	10.9 ± 0.73	8.9 ± 0.29	16.64 ± 2.36	70.2 ± 0.14	54.15 ± 0.51	112.5 ± 3.19
IC281987	12.4 ± 0.41	12.8 ± 4.54	15.1 ± 0.54	26.1 ± 0.41	41.54 ± 0.16	59.58 ± 0.12
T-9	9.06 ± 0.46	10.5 ± 0.51	10.1 ± 0.27	82.7 ± 4.88	122.66 ± 0.85	143.91 ± 1.02
	F value	CD (0.05)	CD (0.01)	*F* value	CD (0.05)	CD (0.01)
CO_2 conc.	*1.69*	*NS*	*NS*	*477.45***	*0.031*	*0.042*
Genotypes	*13.79***	*1.49*	*1.99*	*773.46***	*0.051*	*0.068*
CO_2 conc. ×Genotypes	*1.01*	*NS*	*NS*	*221.45***	*0.088*	*0.118*

** Significant at *P*< 0.01.

ratio by 10-88%. Different CO_2 concentrations (550 and 700 µmol mol^{-1}) caused significant variations (P< 0.01) in sucrose/starch ratio among genotypes, amongst which the genotype IC436720 showed a lower ratio than the others. Ambient CO_2 level is best state for highest sucrose/starch ratios (IC587753, IC436720, IC519805, IC343952, IC281987 and T-9), though, IC282009 and IC436610 reached their highest levels of sucrose/starch ratio only at elevated conditions (Table 3).

DISCUSSION

Carbohydrates are the primary molecules to provide energy, and act as primary messengers for plant growth, development and other physiological processes. Under elevated CO_2 conditions, increased carbohydrate contents in plant tissue affect repression of genes, encoding the expression of rubisco and other photosynthetic proteins (Van Oosten and Besford, 1996). Glucose, fructose and sucrose are the major molecules that regulate photosynthesis and participate in carbohydrate signaling. Among these carbohydrate molecules, sucrose cycling is a key path for carbohydrate signaling. At elevated CO_2, carbohydrates accumulated in plant tissues as their consumption was lower than the production. The results also showed rise in leaf carbohydrate content at elevated atmospheric CO_2 concentrations.

The major products of carbohydrate metabolism are sucrose and starch and the regulation of these metabolites is in strict control of different enzymes like SuSy, invertase and SPS. Sucrose synthesis is generally considered to be catalyzed by SPS, whereas sucrose breakdown is largely catalyzed by SuSy and Invertases. SuSy plays both roles of sucrose synthesis and breakdown. This enzyme is homologous to SPS which catalyzes the penultimate step in sucrose synthesis. The utilization of sucrose depends on its breakdown into hexose through SuSy and invertase. SuSy converts sucrose into UDPG and fructose in the presence of UDP, inversely it can synthesize sucrose from UDPG and fructose whereas, invertase (hydrolase), cleave sucrose into glucose and fructose. Plant Invertases: (I) acid invertase (extracellular/cell wall invertase) cleave sucrose most efficiently between pH 4.5 and 5.0 (II) neutral invertase (cytoplasmic invertase) of pH optima for sucrose cleavage in the neutral range.

Table 3. Hexose/Sucrose and Sucrose/Starch ratios for *Vigna mungo* genotypes at different CO_2 concentrations.

Genotypes	Hexose/Sucrose			Sucrose/Starch		
	CO_2 concentration (µmol mol^{-1})					
	380	550	700	380	550	700
IC587753	1.08 ± 0.132	1.65 ± 0.227	1.30 ± 0.003	0.29 ± 0.004	0.20 ± 0.001	0.28 ± 0.020
IC436720	0.86 ± 0.012	0.94 ± 0.042	1.18 ± 0.164	0.12 ± 0.008	0.08 ± 0.002	0.09 ± 0.010
IC519805	1.26 ± 0.052	1.38 ± 0.050	0.83 ± 0.039	0.14 ± 0.009	0.12 ± 0.016	0.11 ± 0.008
IC343952	0.83 ± 0.020	0.93 ± 0.047	1.12 ± 0.007	0.11 ± 0.004	0.09 ± 0.003	0.09 ± 0.006
IC282009	0.98 ± 0.024	1.02 ± 0.004	0.98 ± 0.015	0.18 ± 0.009	0.28 ± 0.008	0.55 ± 0.022
IC436610	0.87 ± 0.022	0.91 ± 0.012	1.64 ± 0.318	0.15 ± 0.010	0.16 ± 0.004	0.15 ± 0.017
IC281987	1.11 ± 0.028	1.56 ± 0.433	1.35 ± 0.093	0.48 ± 0.023	0.31 ± 0.110	0.25 ± 0.010
T-9	1.05 ± 0.047	1.42 ± 0.023	1.16 ± 0.002	0.11 ± 0.001	0.09 ± 0.004	0.07 ± 0.001
	F value	CD (0.05)	CD (0.01)	*F* value	CD (0.05)	CD (0.01)
CO_2 conc.	*22.57***	*0.072*	*0.095*	*5.83***	*0.021*	*0.028*
Genotypes	*13.59***	*0.117*	*0.156*	*81.15***	*0.034*	*0.046*
CO_2 conc.×Genotypes	*10.31***	*0.202*	*0.270*	*17.14***	*0.059*	*0.079*

** Significant at *P*< 0.01.

Marked differences in acid invertase and neutral invertase activity were observed with high acid invertase activity being observed over the neutral one indicating a possible role for acid invertase in sucrose breakdown. The difference in the activities between acid and neutral invertase may be because of the marked differences between the catalytic sites. Loss of enzyme activity following tissue homogenization and strong inhibition by glucose and fructose are the main reasons for the low catalytic efficiency of neutral invertase. However, acid invertase is inhibited by its reaction products, glucose acting as a non-competitive inhibitor and fructose as a competitive one. Higher acid invertase activity throughout the current study suggested that it is the key enzyme in sucrose unloading and as well in the source/sink balance within the plant (Islam and Khan, 2001). Islam *et al*. (2006) reported high neutral invertase activity over acid invertase in tomato plant at elevated CO_2 levels. In the present study *V. mungo* genotypes showed inconsistent response in invertase activity with enhanced CO_2 concentration, activity being decreased for IC587753, IC436720, IC519805 and IC343952, although increased for IC282009, IC436610, IC281987 and T-9. Sucrose content and invertase activity trends confirmed that high sucrose accumulating genotypes possessed low invertase activity, while low sucrose accumulating genotypes maintained high invertase. Similar observations have been reported earlier by Stepansky *et al*. (1999) for *Cucumis melo* genotypes. Varied responses of elevated CO_2 have been reported by Moore *et al*. (1998) for different plant species. Arabidopsis, corn, cotton, cucumber, pea, radish, soybean, spinach, tobacco, tomato and wheat showed decline in invertase activity, however, bean and sunflower showing increase in invertase activity.

Neutral invertases are most probably located in the cytosol like SuSy. This cytoplasmic invertase is most active in the regulation of intracellular glucose and fructose levels in mature tissues over Sucrose Synthase (Van den Ende and Van Laere, 1995). The present study's results are in agreement with this hypothesis showing high invertase activity over SuSy, indicating a very prominent role for invertase in sucrose breakdown and sucrolysis while, SuSy activity was low and may have a secondary role in sucrose synthesis. This is in contrast with the findings of Sung *et al*. (1989) (lima bean) and Riffkin *et al*. (1995) (wheat) with a high SuSy activity over invertase. High SuSy activity under elevated CO_2 concentrations indicated that the rates of sucrose synthesis were higher at 700 μmol mol^{-1}. Jenner and Hawker, (1993) also reported the enhancement of SuSy activities by CO_2 concentration being doubled. The extent of stimulation of SuSy activity depends on species and on environmental conditions.

SPS is a key regulatory enzyme involved in the conversion of photo-assimilate to sucrose in leaves (Huber *et al*., 1989). The activity of SPS has been observed to vary among species and genotypes. Enhancement of activity was recorded for corn, pea, soybean, spinach and sunflower however, in cotton, cucumber, Arabidopsis, bean, tobacco, tomato and wheat, a decline was observed at high CO_2 level (Moore *et al*., 1998). Variation in SPS activity was observed in *V. mungo* genotypes at enhanced CO_2 concentration. The SPS activity increased linearly with CO_2 concentration for IC436720, IC282009, IC436610, and T-9. The increase in SPS activity at elevated CO_2 was also reported by Vu *et al*. (2006) in sugarcane and Vara Prasad *et al*. (2004) for *Phaseolus vulgaris*. SPS activity profile in the present study did not coincide with sucrose pools and varied among genotypes under elevated CO_2 concentrations. The reason for the varied activity could be because of the differential regulation of this enzyme by covalent modification via phosphorylation/dephosphorylation (Huber and Huber, 1996), and via its allosteric effectors glucose-6-P (activator) and Pi (inhibitor) (Stitt *et al*., 1988). Modulation by light activation could be yet another reason for the difference in SPS activity. Huber and Huber (1996) reported that soybean species of class I and II showed little, however, soybean species "Maple Presto" showed significant light activation of SPS. Similarly differences in light activation of SPS activity were also reported among *Nicotiana* species and cultivars of *Nicotiana tabacum*. Transgenic tomato plants expressing maize SPS showed relatively little light modulation (Galtier *et al*., 1995). The basis for the lack of modulation may be because of differences in quaternary structure, which make phosphorylating sites less accessible to protein kinases.

Nonstructural carbohydrates, starch and sucrose, play a major role in plant metabolism. The level of these carbohydrates gets increased at elevated atmospheric CO_2 concentrations (Vu *et al*., 2001). In the present study plant exposed to higher CO_2 concentration of 550 and 700 μmol mol^{-1} exhibited high starch and sucrose content as compared with ambient CO_2 treatments. Starch content was several fold higher than sucrose throughout the study, indicating that it was a major factor in regulation of assimilate partitioning, quite similar to the findings by Katny *et al.* (2005). The increased starch accumulation under elevated CO_2 conditions affects sucrose metabolism and decreases glucose content (Walter *et al*., 2005). The high intercellular sucrose at elevated CO_2 concentration helps in maintenance of cell turgor (osmotic substance). Consequently, sucrose formed under the elevated CO_2 concentration was hydrolyzed to glucose and fructose through invertase and sucrose synthase, resulting in higher hexose contents (Vu *et al*., 2001). This increased hexose level down regulates transcription of photosynthetic gene via sugar sensing and signaling pathway. Increased hexose production may be one of the factors responsible for inhibition of photosynthesis (Pego *et al*., 2000). High fructose content (over glucose) under elevated CO_2 indicated that fructose may be the preferred substrate for respiration. Similarly, Islam *et al.* (2006) also reported high fructose level over glucose at elevated CO_2 for *Lycopersicon* cultivars. In the present study glucose and fructose concentrations for genotypes increased about 20-90% and 10-140%, respectively under elevated CO_2. The increased concentration of glucose significantly affects cell as well as leaf growth, since it is the main plant metabolite, the substrate for respiration and structural essential unit of starch and cellulose synthesis.

Hexoses/sucrose ratio is an important parameter that reflects changes in carbohydrate metabolism and hexokinase activity. Throughout the present study, the hexoses/sucrose ratio at elevated CO_2 concentration was approximately 0.8-1.6, however, at ambient CO_2 concentration it was approximately 1.0, indicating higher hexokinase activity over SPS at high CO_2 concentrations. These results are in tune with the findings of Urbonaviciute *et al.* (2006) for radish at elevated CO_2. High hexose/sucrose ratio confirmed high Invertase activity over SPS, SuSy and indicated the rate of sucrose breakdown to be greater than synthesis at elevated CO_2. Enzyme activity patterns of SuSy, Invertase and SPS validated these findings. Genotypes (IC436720, IC519805, IC343952, IC282009) showed low hexose/sucrose ratios representing high CO_2 assimilation along with high sucrose formation, indicating that these genotypes are more tolerant to elevated CO_2 for carbon partitioning and carbohydrate metabolism. The difference in hexose/sucrose ratio observed in the present investigation may be because of substantial differences in acid invertase activity in mature leaves among the genotypes. No direct relationship was observed between invertase activity and hexose concentrations, however, an inverse relationship was observed between invertase activity and in leaf sucrose concentration. Sucrose accumulating genotypes (IC587753, IC436720, IC519805, IC343952) had low acid invertase activity. Conversely, genotype (IC282009) not accumulating sucrose revealed high activities of invertase under elevated CO_2 conditions. These results suggest that sucrose accumulation may be prevented as a result of hydrolysis through high activities of acid invertase. Interesting observations were apparent in IC436610, IC281987 and T-9 having high sucrose content along with high invertase activity. The reason for accumulation of sucrose in these genotypes may be because of the rate of sucrose formation being more than its degradation.

The foliar sucrose/starch ratio has been used as an indicator of photo-assimilate allocation as well as strong correlation between SPS activity and sucrose/starch ratio in leaves. The decrease in sucrose/starch ratio (because of the low SPS activity) was mainly due to the increase in starch content. In the present study sucrose/starch ratio was lower in the leaves of plants grown in high CO_2 mediums than those grown under ambient conditions because of starch accumulation. These results are in conformity with those of Signora *et al*. (1998) for *Arabidopsis thaliana*. Large genotypic variations in the relative quantities of sucrose and starch accumulation were observed among the studied genotypes. The difference in starch accumulation was proposed as a potential basis for explaining genotypic variations through feedback regulation of SPS. Constitutive enhancement of SPS activity therefore has an important implication for source/sink relationship and as well for carbon metabolism. Starch synthesis is promoted when starch serves as a transient sink to accommodate excess photosynthate which cannot be converted to sucrose to be exported. The accumulation of large starch grains under elevated CO_2 conditions could physically disrupt the chloroplast. Alternatively the rate of photosynthesis under elevated CO_2 levels may become limited by the rate at which newly fixed carbon is converted into starch and sucrose. Genotypes showed increase in carbon partitioning to sucrose with simultaneous decrease in starch accompanied by high SPS activity. A plant with decreased capacity of starch synthesis will be less susceptible to inhibition due to physical effects of starch grain while more susceptible to inhibition due to an inadequate capacity for the synthesis of carbohydrates. The capacity of starch synthase under high CO_2 concentrations enables plants to achieve high rates of photosynthesis (Murchie *et al*., 1999). Growth at elevated CO_2 levels always leads to starch accumulation by increasing the expression of AGPase. This increased expression of AGPase will not only allow increased accumulation of carbohydrates but may also allow higher rate of photosynthesis under elevated CO_2 conditions (Ludewig *et al.*, 1998). From this viewpoint, genotypes which are more active in starch metabolism are more likely of the chance to experience sink limited growth condition as often occurring at high CO_2 levels.

In conclusion, CO_2 enrichment up-regulated the activities of sucrose metabolizing enzymes, resulting in greater accumulation and export of carbohydrates associated with photosynthetic activities. It was observed that changing CO_2 levels led to alterations in sucrose metabolism which were not associated with sucrose pool sizes. Switching to high CO_2 significantly triggered a rapid increase of SPS activity in leaves. Enhanced SPS activity at high CO_2 level played a major role in determining the sucrose supply to growing sinks. The differential response of elevated CO_2 was observed among genotypes. High SPS levels synthesized sucrose molecules, ultimately being converted to hexose or starch depending upon the activity of hexokinase or starch synthase. The activity of these enzymes varied among genotypes. A consistent correlation was observed between SPS activity and carbohydrate metabolism. Enhancement of SPS and SuSy activity *vs*. decrease in invertase activity, at elevated CO_2 conditions, increased sucrose level, maintaining a low level of hexose/sucrose ratio. This low hexose/sucrose ratio could end up with an activation of photosynthetic genes without any involvement of hexose molecules into sucrose cycling. Decreased levels of sucrose/starch may stimulate the conversion of photo-assimilates into starch at sink site. This high starch level might lead to enhancement of photosynthesis at elevated CO_2. In this case, low hexose/sucrose and sucrose/starch ratios enhanced the photosynthetic ability coupled with higher efficiency of carbon partitioning for genotype IC436720 as compared with other genotypes. The up-regulation of leaf carbohydrate metabolizing enzymes indicated the genotype's further acclimation at elevated CO_2 resulting in enhanced growth and productivity of the genotype IC436720.

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کربوهیدراتها و آنزیمهای سوخت و ساز ساکاروز موجود در برگ ژنوتیپهای vigna mungo تحت تأثیر کازگربنیک به مقدار زیاد

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چکیده

تأثیر غلظتهای متفاوت گاز کربنیک بر روی آنزیمهای سوخت و ساز ساکارز و بر روی متابولیسم کربوهیدراتها در مورد هشت ژنوتیپ بلک گرم blackgram (vigna mungo L. Hepper) که در اطاقک‌های روباز تحت شرایط گاز کربنکی محیط (۳۸۰ µmol mol^{-1}) در برابر گاز کربنیک افزایش یافته در سطوح ۵۵۰ و ۷۰۰ (µmol mol^{-1}) مورد بررسی قرار گرفتند. فعالیت بیشتر اینورتاز اسیدی (acid invertase) نسبت به اینورتاز خنثی (neutral invertase) نشان‌دهندهٔ نقش عمدهٔ اینورتار اسیدی در شکسته شدن ساکارز بود. فعالیت بیشتر اسید اینورتاز نسبت به ساکارز سینتاز Suerose Synthase (Susy) دلالت داشت بر نقش اساسی اینورتاز در شکسته شدن و تجزیه ساکارز فعالیت سینتاز فسفات ساکارز Suerose Phosphate Synthase (SPS) با اندازهٔ نقاط تجمع ساکارز موجود در برگهای بالغ مطابقتی نداشت بلکه در بین ژنوتیپها متفاوت بود. گیاهانی‌که در معرض گاز کربنیک بالا قرار گرفته بودند. دارای محتوای ساکارز و نشاستهٔ بیشتری، نسبت به گیاهان در معرض گاز کربنیک معمول محیط، بودند. نشاستهٔ موجود در برگ که در تمام طول تحقیق چندین برابر ساکارز موجود در برگ بود نشاندهندهٔ نقش اساسی این ترکیب در تنظیم تفکیک مواد اسیمیله (Assimilate) بود. افزایش غلظت گلوکز در مقایسه با فروکتوز (در مورد ژنوتیپهای پرورش یافته در شرایط گاز کربنیک زیاد) به ترتیب در دامنه‌های ۲۰ تا ۹۰ درصد و ۱۰ تا ۱۴ درصد قرار داشت. نسبت هگزوزها به ساکارز (hexoses/ suerose) در مورد محیط با گاز کربنیک بالا در فاصلهٔ ۰/۸ تا ۱/۶ قرار داشت در حالیکه در مورد گاز کربنیک معمول محیط تقریباً معادل واحد بود. از ژنوتیپ‌های (IC 4636720, IC529508,IC343952 , IC282009) که دارای نسبت‌های پائین هگزوز به ساکارز (hexose/ sucrose) بودند. (نمایانگر جذب و هضم بالای گاز کربنیک همراه با تشکیل به مقدار زیاد ساکارز) چنین برداشت می‌شد که دارای تاب و تحمل بیشتری (در محیط گاز کربنیکی بالا) در زمینهٔ جداسازی کربن و ساختن کربوهیدراتها هستند. تنظیم آنزیمهای مؤثر در ساخت کربوهیدراتها در برگ (دارای نسبت پائین هگزوز به ساکارز و همچنین نسبت پائین ساکارز به نشاسته در مورد ژنوتیپ IC436720 (در قیاس با سایر ژنوتیپ‌ها) باعث تقویت توان فتوسنتزی این ژنوتیپ گردیده که همراه با بازده بیشتر جداسازی کربن (نشان‌دهندهٔ تطبیق بیشتر این ژنوتیپ با محیط دارای گاز کربینک بالا) می‌توان دلیل مفید فایده بودن این ژنوتیپ در ارتباط با رشد بالا و محصول دهی فراوان آن در شرایط آب و هوائی دچار تغییر در آینده باشد.