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Elevated Atmospheric CO_2 Concentration Enhances Carbohydrate Metabolism in Developing *Lycopersicon esculentum* Mill. Cultivars

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ABSTRACT

This study examined the influence of preharvest elevated atmospheric CO_2 concentration (850 ± 50 ppm) on the fruit growth, total soluble solids (TSS), fructose, glucose, sucrose, soluble and cell wall-bound acid invertase (AI) (ß-fructofuranoside fructohydrolase, EC 3. 2. 1. 26), sucrose synthase (SuSy) (UDP glucose: D-fructose 2 -glucosyltransferase, EC 2. 4. 1. 13) and sucrose phosphate synthase (SPS) (UDP glucose: D-fructose-6 -phosphate 2 -glucosyltransferase, EC 2. 4. 1. 14) activities of domesticated tomatoes (*Lycopersicon esculentum* Mill. cv. 'Lady First', 'Momotaro' & 'Minicarol') during fruit development. Tomato plants grown in CO_2-enriched conditions accumulated more carbohydrates in their fruit and exhibited significantly larger fruits compared to the control (350 ± 50 ppm). The CO_2-enriched tomato fruit contained significantly higher concentrations of TSS, fructose and glucose during development. The amount of reducing sugars increased at the advancement of maturity, with fructose being the predominant sugar. The treated fruit showed significantly higher activity of soluble AI and SuSy activities compared to the control. There were no significant differences in sucrose concentration and SPS activity between the treatments. The results indicate that enriched CO_2 treatment enhances fruit growth, and improved the carbohydrate contents of tomato fruit compared to fruits exposed to ambient CO_2 concentration, which might be due to enhanced translocation of photosynthate with elevated enzyme activities in the CO_2-enriched treatment.

Key Words: Carbon dioxide; Sugar; Enzyme; Metabolism; Tomato

INTRODUCTION

CO_2 enrichment is commonly practiced in the cultivation of green-house crops for it increases both yield and profit. The atmosphere around plants normally contains about 350 - 400 ppm of CO_2. At this level a plant must process a large volume of air to obtain sufficient CO_2 for plant growth and development and for synthesis of the products stored in such organs as fruits, leaves, stems and roots. Numerous experimental studies have been conducted to investigate the effect of elevated atmospheric CO_2 concentration on economically important agricultural crops (Kimball & Idso, 1983; Kimball *et al*., 1995; Pinter *et al*., 1996; Clark *et al*., 1999; DeLuis *et al*., 1999). Plant responses to elevated CO_2 are fundamentally medicated by photosynthesis (Drake *et al*., 1997; Norby *et al*., 2001), and can potentially lead to a suite of morphological and growth changes. Since the concentration of CO_2 is so small, crop plants will grow more rapidly and produce higher yields, possibly with improved quality, if the amount of available CO_2 is increased. The favorable response to CO_2 enrichment might be due to increased sugar production thereby triggering some biochemical changes. Lilley *et al.* (2001) reported that elevated CO_2 conditions produced an average increase in total non-structural carbohydrate contents of 28% for clover and 16% for phalaris. The sugar content of tomato fruit is a key determinant of quality and value of the crop, whether it is for the fresh produce or for processing. Furthermore, soluble sugar levels contribute strongly to the tomato flavor and to soluble solids content (Stevens *et al*., 1977). Therefore, increasing these levels has been the goal of many research efforts. Sucrose is the major sugar form in which carbohydrate in transported in the tomatoes. In sink tissues, un-loaded sucrose has to be hydrolyzed for metabolism. Depending on the plant species, sucrose can be hydrolyzed either by invertase or by SuSy. Several authors suggested that the sucrose concentration of tomato fruit was determined by AI activity (Ohyama *et al*., 1995; Klann *et al*., 1996; Scholes *et al*., 1996) and SuSy activity (Wang *et al*., 1993; N'tchobo *et al*., 1999; Islam, 2001). But, transformed tomato fruit with increased SPS activity showed increased sucrose turnover, suggesting that SPS activity may be a limiting step in sucrose synthesis in tomato fruit (Nguyen-Quoc *et al*., 1999). Laporte *et al.* (1997) reported that increased leaf SPS activity result increased fruit sugar content, and SPS over expression in tomato leaves results in decreased starch accumulation in plant grown with CO_2 enrichment (Murchie *et al*., 1999). Micallef *et al*. (1995) examined that altering starch/sucrose partitioning by increasing the capacity for sucrose synthesis can affect acclimation to elevated CO_2 partial pressure and flowering and fruiting in transgenic tomatoes.

The beneficial effects of CO_2 enrichment with regard to yield, morphological and physiological parameters have been researched extensively but little information establishes a relationship between CO_2 enrichment and carbohydrate metabolism in developing tomato fruit. The objective of the study was to investigate the effect of CO_2 enrichment on the sugar accumulation and their metabolism in developing tomato cultivars.

MATERIALS AND METHODS

Plant materials and cultural methods. The tomato cv. 'Momotaro', 'Lady First' and 'Minicarol' were used in the study (Table I). The seeds were sown in nutrient film technique (NFT) channels in the green-houses. The electrical conductivity (EC) and pH of the nutrient solutions were maintained at 1.3 - 1.5 $mS.cm^{-1}$ and 5.5 - 6.5, respectively. The concentrations (mm) of basic elements in the solution were: NO_3^- = 8.0; $H_2PO_4^-$ = 0.7; SO_4^{2-} = 1.0; K^+ = 4.0; Ca^{2+} = 2.0; Mg^{2+} = 1.0. The air was enriched with CO_2 by supplying liquid CO_2 through computer controlled magnetic valve connected to an infrared gas analyzer. Application of elevated CO_2 began immediately after crop establishment and continued until the end of sampling. During daytime (6.00 to 18.00) the CO_2 enriched green-house contained 850 ± 50 ppm CO_2, while the control had 350 ± 50 ppm with a ventilating temperature of 27°C. After anthesis, each flower was tagged and treated with 4 -chlorophenoxy acetic acid for uniform fruit setting. All cultivars were harvested at: (i) Mature green, a completely green skin that will turn red either on or off the vine, (ii) Pink, 40 - 50% pink or red skin, and (iii) Red, > 90% fully red but firm. Fifteen fruits each day were selected randomly and three replicates of five fruits each were picked at random from the 15 fruits for biochemical and enzymatic analysis.

Carbohydrate determination. Soluble sugars were analyzed using a high-performance liquid chromatograph (HPLC) as described in the previous paper (Islam, 2001). The TSS concentration of the fruit juice was determined with a digital refractometer.

Extraction and assay of enzymes. The extraction and assay of all enzymes were conducted as described by Islam (2001). For SPS and SuSy, a 5 g sample of fruit tissues were ground in a cooled mortar and pestle with 10 mL of 0.2 m K-phosphate buffer (pH 7.8) containing 10 mm ascorbate, 15 mm $MgCl_2$, 1 mm EDTA (ethylenediaminetetraacetic acid) and 1 mm dithiothreitol (DTT), 20 mm 2 –mercaptoethanol, 5% (v/v) glycerol, 10% (w/v) insoluble PVPP (polyvinylpolypyrrolidone), and 1% Dowex- 1 (chloride form). The resulting homogenate was filtered through four layers of cotton cloth and the filtrate was centrifuged at 10,000 X g for 20 min. The supernatant was dialyzed with 5 mm of 0.2 m K-phosphate buffer (pH 7.8) for 24 h and the inner solution was used as the crude enzyme. SPS and SuSy activity were assayed at 37°C by the method described by Nascimento *et al.* (1997) with slight modification. Reaction mixtures (70 µL) contained 50 mm HEPES-NaOH buffer (pH 7.5), 15 mm NaF, 15 mm $MgCl_2$, 25 mm fructose- 6P, 25 mm glucose- 6P and 25 mm UDP (uridine 5'-diphosphate) -glucose. The mixtures were incubated for 30 min at 37°C and incubation was terminated with the addition of 70 µL of 30% KOH. Enzyme blanks were terminated with KOH at 0 min. Tubes were heated at 100°C for 10 min to destroy any unreacted fructose or fructose- 6P. After cooling, 2 mL of mixture of anthrone reagent (150 mg anthrone with 100 mL of 15% H_2SO_4) was added and the mixture incubated in a 40°C water bath for 15 - 20 min. After cooling, color development was measured at 620 nm. SuSy was assayed as above but with 25 mm fructose instead of fructose- 6P, and in absence of glucose-6P. The protein concentration in enzyme solutions was determined according to the method of Lowry *et al.* (1951) using crystalline bovine serum albumin (BSA) as a primary protein standard. The enzyme activity was measured as the amount of sucrose or sucrose-P produced per min per mg protein. The experiments were carried out at 0 - 4°C.

Statistics. A randomized complete block design with three replications was used. Data were analyzed using analysis of variance (ANOVA), and the level of significance was calculated from the *F* value of ANOVA.

RESULTS AND DISCUSSION

Fruit growth (g) and TSS (%). The effect of CO_2 enrichment on fruit growth during development is presented in Fig. 1. There were significant ($P < 0.001$) difference among the cultivars in case of fruit weight at various stages of maturity. 'Lady First' had the highest fruit weight followed by 'Momotaro'. The small fruited 'Minicarol' produced the smallest fruit. Furthermore, tomato plants grown in elevated CO_2 produced significantly (**$P < 0.01$) larger fruits compared to the control groups at various stages of maturity (Fig. 1). Several authors (Kimbal & Mitchell, 1979; Kawashima *et al*., 1993; Islam *et al*., 1995; Bhboudian & Colin, 1995) reported that CO_2 enrichment resulted in the production of heavier fruits as found in the present study. The plants probably accumulated more carbohydrates in their fruits because of the higher rates of photosynthesis in the CO_2 -enriched environment, which caused the larger fruits. The CO_2 -enriched tomatoes had a significantly (*$P < 0.05$) higher percentage of TSS compared to control fruits in all cultivars studied (Fig. 2). This was consistent with the findings of Behboudian and Colin (1995), who reported increases in TSS concentration with preharvest exposure of plants to 1000 ppm CO_2 compared to ambient CO_2 (340 ppm) fruit.

Soluble sugars (mg/g fresh weight). Table II shows the influence of CO_2 on the soluble sugar concentrations during fruit development. Fructose (**$P < 0.01$) and glucose (**$P < 0.01$) concentrations were significantly higher in the CO_2 -enriched fruits than in the control in all the cultivars at all

Table I. Features of the *Lycopersicon esculentum* cultivars used in this study

Cultivars	Breeding Company	Color	Growth habit	Fruit shaped	No. of locule	No. of flower per truss	Truss type
Momotaro	Takii seed co.	Red	Indeterminate	Round	5-6	5-7	Single
Lady First	Aisan seed co.	Pinky red	Indeterminate	Heart	10-12	10-15	Double
Minicarol	Sakata seed co.	Deep red	Indeterminate	Cherry	2	20-30	Double/ Multi

Table II. Effect of preharvest elevated atmospheric CO_2 concentration on the soluble sugar contents (mg/g fresh weight) in different tomato cultivars at various stages of maturity

Cultivars and Treatments	Sucrose			Glucose			Fructose		
	MG	Pink	Red	MG	Pink	Red	MG	Pink	Red
MT (CO_2 enriched)	0.21 ± 0.02*	0.12 ± 0.01	0.04 ± 0.01	11.18 ± 0.18	14.28 ± 0.16	19.03 ± 0.12	12.08 ± 0.10	15.40 ± 0.12	21.30 ± 1.27
MT (Control)	0.15 ± 0.01	0.07 ± 0.00	0.01 ± 0.00	9.39 ± 0.10	11.36 ± 0.10	14.03 ± 0.66	10.40 ± 0.12	12.70 ± 0.21	14.90 ± 0.70
LF (CO_2 enriched)	0.18 ± 0.01	0.11 ± 0.00	0.02 ± 0.00	9.85 ± 0.13	11.35 ± 0.19	15.60 ± 0.35	11.10 ± 0.12	13.19 ± 0.10	15.83 ± 0.21
LF (Control)	0.16 ± 0.01	0.04 ± 0.01	0.00 ± 0.00	8.99 ± 0.06	9.88 ± 0.07	11.70 ± 0.50	9.10 ± 0.05	10.75 ± 0.10	11.73 ± 0.50
MC (CO_2 enriched)	0.39 ± 0.01	0.27 ± 0.00	0.22 ± 0.01	11.47 ± 0.17	14.30 ± 0.25	16.37 ± 0.32	11.46 ± 0.26	14.81 ± 0.13	19.06 ± 0.31
MC (Control)	0.29 ± 0.00	0.23 ± 0.01	0.16 ± 0.01	10.85 ± 0.36	13.15 ± 0.28	15.00 ± 0.44	10.97 ± 0.23	13.21 ± 0.25	17.77 ± 0.81

*Average of three replication (each replicate composites of 5 fruits) ± standard error; MG= Mature green; MT= Momotaro; LF= Lady First; MC= Minicarol

stages of maturity. The reducing sugar concentrations increased more rapidly at the later stages of fruit development than early stages. The concentration of fructose and glucose were always much higher than those of sucrose (Table II). Reducing sugars accounted for more than 95% of the total soluble sugar, and the concentration of fructose was higher than that of glucose. The hexose sugars, fructose and glucose, make up 50% of the dry matter of the mature fruit and accounted for more than 95% of the total soluble sugar during fruit development (Davies & Hobson, 1981), which corroborated the present findings. During the early stages of fruit development the sucrose levels peaked, and then declined sharply. Miron and Schaffer (1991) and Islam and Khan (2001) reported that high amounts of sucrose did not accumulate in tomato fruits at the later stages. In the early stage of tomato fruit development sucrose is accumulated, but after that, it is hydrolyzed to fructose and glucose by the sucrose cleavaging enzyme(s) probably mediated mainly by the action of AI (Ho, 1996; Klann *et al*., 1996; Koch, 1996; Schaffer *et al*., 1999). Hence, the concentrations of reducing sugars are higher than the sucrose. This enzyme is widespread in fruit and often increases in activity during ripening. However, the activity is not always associated with increased metabolism of sucrose. For instance, in tomato fruit this enzyme increases in activity, even though this fruit contains no sucrose (Ho, 1996). This suggests that during early stage of fruit development, the rapid cell growth as well as other energy metabolism processes of fruit may utilize the hydrolyzed sugars. Subsequently, during the later stage of fruit development, cell growth and other energy requirements for he fruits are minimal, so the accumulation of fructose and glucose ultimately are much higher than that of sucrose. The CO_2 -enriched fruit accumulated higher sugar contents than did the control fruit. We have monitored that (data were not shown), in all cultivars, there was no significant difference between the CO_2 -enriched and control plants in case of days to first flower bud appearance and days to first anthesis. Therefore, the changes in fruit sugar levels were the specific effect of CO_2 treatment on carbohydrate metabolism not a result of general advancement in

Fig. 1. Effect of CO_2 enrichment on the fruit fresh weight (g) of tomato cultivars at various stages of maturity. Data are means of 3 replicates composed of 5 fruit each. Vertical bars represent standard error of the mean. MT = 'Momotaro', LF = 'Lady First', MC = 'Minicarol'

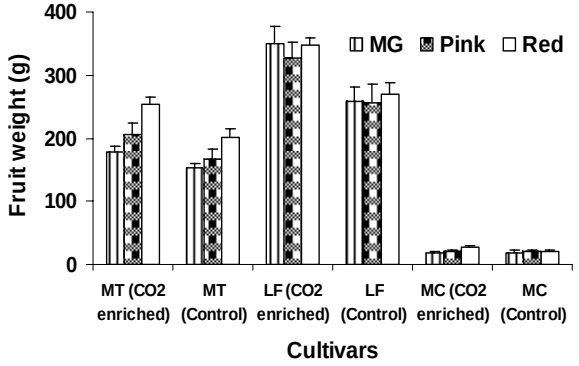
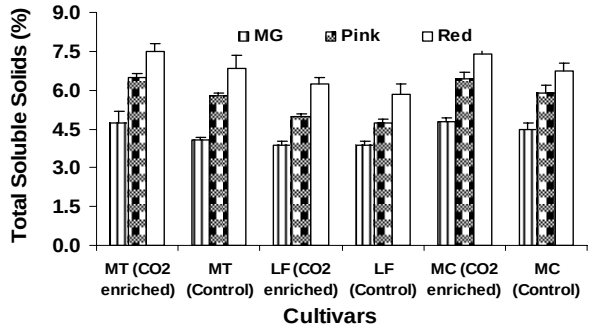


Fig. 2. Effect of CO_2 enrichment on the total soluble solids (%) of tomato cultivars at various stages of maturity. Data are means of 3 replicates composed of 5 fruit each. Vertical bars represent standard error of the mean. MT = 'Momotaro', LF = 'Lady First', MC = 'Minicarol'



physiological age of the fruit. Lilley *et al*. (2001) reported that elevated CO_2 conditions produced an average increase in total nonstructural carbohydrate contents of 28% for clover and 16% for phalaris. These were possibly due to increased photosynthesis in the CO_2 -enriched plants, resulting in higher translocation of more photosynthate into

Fig. 3. Effect of CO_2 enrichment on the changes in soluble acid invertase activities (μ mole/min/mg protein) of tomato cultivars at various stages of maturity. Data are means of 3 replicates composed of 5 fruit each. Vertical bars represent standard error of the mean. MT = 'Momotaro', LF = 'Lady First', MC = 'Minicarol'

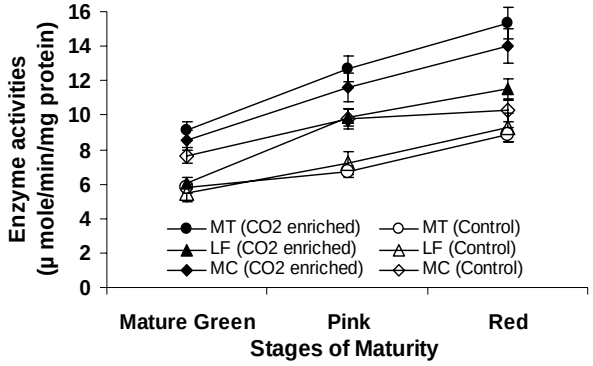


Fig. 4. Effect of CO_2 enrichment on the changes in cell wall-bound acid invertase activities (μ mole/min/mg protein) of tomato cultivars at various stages of maturity. Data are means of 3 replicates composed of 5 fruit each. Vertical bars represent standard error of the mean. MT = 'Momotaro', LF = 'Lady First', MC = 'Minicarol'

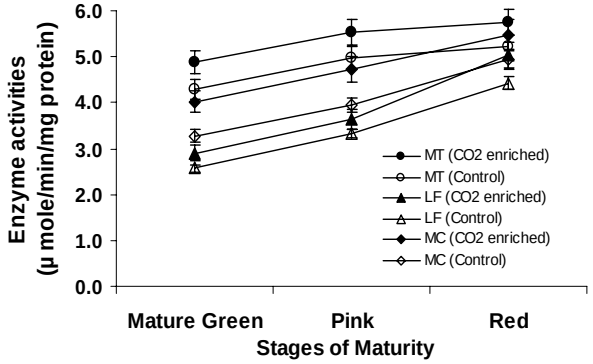


Fig. 5. Effect of CO_2 enrichment on the changes in sucrose synthase (SuSy) activities (μ mole/min/mg protein) of tomato cultivars at various stages of maturity. Data are means of 3 replicates composed of 5 fruit each. Vertical bars represent standard error of the mean. MT = 'Momotaro', LF = 'Lady First', MC = 'Minicarol'

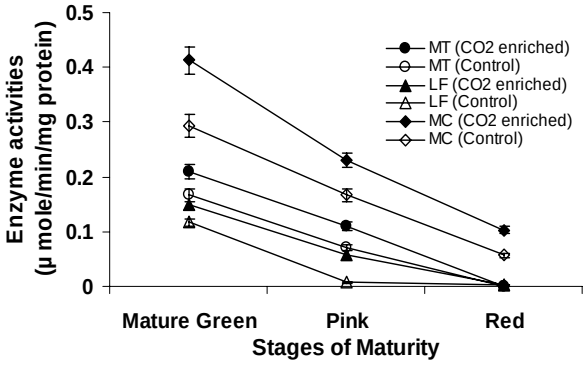
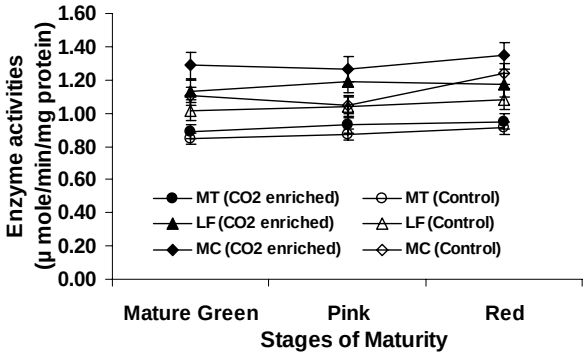


Fig. 6. Effect of CO_2 enrichment on the changes in sucrose phosphate synthase (SPS) activities (μ mole/min/mg protein) of tomato cultivars at various stages of maturity. Data are means of 3 replicates composed of 5 fruit each. Vertical bars represent standard error of the mean. MT = 'Momotaro', LF = 'Lady First', MC = 'Minicarol'



the fruit and increased sugar concentration.

Enzyme activities (μ mole/min/mg protein). Soluble AI activity was predominant in all cultivars studied and significantly higher soluble AI activity was observed in the CO_2 -enriched fruits compared to control (**$P < 0.01$) (Fig. 3). A sharp increase in soluble AI activity was observed from mature green stage until the red ripe stage. No significant differences in cell wall-bound AI activity were observed between the treatments (Fig. 4). This pattern is similar to other studies on tomato (Miron & Schaffer, 1991; Islam & Khan, 2001). Fig. 5 shows the effect of CO_2 enrichment on SuSy activity in different tomato cultivars. In all cultivars, SuSy activity was found significantly (**$P >$ 0.01) higher at mature green stage of fruit development, and a rapid decline afterward. The CO_2 enriched fruits showed higher activity than control. The decrease in SuSy activity coincided with the increase in reducing sugar levels (Fig. 5 & Table II). The changes in SuSy activity had almost the same pattern of changes in sucrose concentration during fruit development. There were no significant differences in SPS activity between the treatments (Fig. 6), but CO_2 -enriched tomatoes always showed higher SPS activity than the control. The SPS activity remained relatively constant and did not change drastically throughout fruit development. Nguyen-Quoc *et al*. (1999) reported that in transformed tomato fruit, the increased SPS activity showed increased sucrose turnover, and the increased leaf SPS activity resulted increased fruit sugar content (Laporte *et al*., 1997). Murchie *et al*. (1999) reported that starch accumulation was dramatically increased in tomato plant subjected to CO_2 enrichment but the CO_2 -dependent increase in foliar starch accumulation was much lower in the leaves of SPS transformants than in those of the un-transformed controls in the same conditions. In transgenic tomato plants altering starch/sucrose partitioning by increasing the capacity for sucrose synthesis can affect acclimation to elevated CO_2 partial pressure and flowering and fruiting in tomato (Micallef *et al*., 1995).

It is apparent that CO_2 enrichment increases fruit growth, improves the carbohydrate content, and enhances the enzyme activities of tomato fruit, compared to fruits exposed to ambient CO_2 concentration, which might be due to enhanced translocation of photosynthate with elevated enzyme activities in the CO_2 -enriched treatment. Therefore the regulation of membrane transport of sugars by elevated CO_2 may play a significant role in the import of assimilate. The information obtained in this study regarding the mechanisms through, which carbon dioxide affects various biochemical processes in tomatoes during fruit development is not exhaustive. Further studies are necessary on the mechanisms of atmospheric CO_2 regulation of other metabolic processes such as degradation of cell wall membranes, which would ultimately affect the texture. Further, using new molecular biology techniques that are currently available, studies should be undertaken in an attempt to elucidate how atmospheric CO_2 regulates the various biochemical and physiological processes of tomatoes.

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