The effect of external CO₂ concentration on the expression of carbonic anhydrase (CA) and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) was examined in pea (Pisum sativum cv Little Marvel) leaves. Enzyme activities and their transcript levels were reduced in plants grown at 1000 µL/L CO₂ compared with plants grown in ambient air. Growth at 160 µL/L CO₂ also appeared to reduce steady-state transcript levels for rbcS, the gene encoding the small subunit of Rubisco, and for ca, the gene encoding CA; however, rbcS transcripts were reduced to a greater extent at this concentration. Rubisco activity was slightly lower in plants grown at 160 µL/L CO₂, and CA activity was significantly higher than that observed in air-grown plants. Transfer of plants from 1000 µL/L to air levels of CO₂ resulted in a rapid increase in both ca and rbcS transcript abundance in fully expanded leaves, followed by an increase in enzyme activity. Plants transferred from air to high-CO₂ concentrations appeared to modulate transcript abundance and enzyme activity less quickly. Foliar carbohydrate levels were also examined in plants grown continuously at high and ambient CO₂, and following changes in growth conditions that rapidly altered ca and rbcS transcript abundance and enzyme activities.

As atmospheric levels of CO₂ continue to increase, there is considerable research interest in the mechanisms by which plants respond to changes in CO₂ concentration, and, in particular, how these changes have an impact on photosynthetic processes (see reviews by Bowes, 1991; Stitt, 1991; Sage, 1994; Webber et al., 1994). Many laboratory studies using growth chamber- or glasshouse-grown plants have shown that some species experience a short-term stimulation followed by a decline in photosynthetic rate after prolonged exposure to high-CO₂ (Stitt, 1991; Webber et al., 1994). This decline in photosynthesis has been associated with a reduction in Rubisco activity, although the capacity of the plant for RuBP/Pi regeneration may also limit photosynthetic rate. Studies have shown that Rubisco protein levels are lower in high-CO₂-grown plants, particularly when additional factors such as nutrient limitation, developmental stage, or root-growth space inhibit effective carbohydrate translocation from leaves into sink tissues (Sage et al., 1989; Besford et al., 1990; Rowland-Bamford et al., 1991). These additional factors are an important consideration, since some plant species grown in nonlimiting environments at high-CO₂ concentrations do not exhibit a reduction in photosynthetic capacity (Sage, 1994). Nonetheless, the observed reduction in photosynthetic capacity when source-to-sink translocation is constrained indicates that additional regulatory steps in photosynthesis are operating.

The enzyme CA (EC 4.2.1.1), which is localized primarily in the chloroplast stroma of C₃ higher plants, is thought to play a role in photosynthesis by facilitating diffusion into and across the chloroplast, as well as by catalyzing HCO₃⁻ hydration to supply CO₂ for Rubisco (Badger and Price, 1994). Therefore, it would be anticipated that this enzyme would respond to changes in ambient levels of CO₂ in a manner similar to Rubisco. Previous studies have shown that CA activities are reduced in leaves of Cucumis sativus (Peet et al., 1986), Avena sativa (Cervigni et al., 1971), Gossypium hirsutum (Chang, 1975), and Phaseolus vulgaris (Porter and Grodzinski, 1984) grown at elevated CO₂ concentrations, although the mechanisms of regulation were not identified. Recently, in contrast with earlier studies showing a CO₂-induced reduction in CA activity, it was reported that chloroplast CA mRNA abundance increased during acclimation of Arabidopsis thaliana to elevated CO₂ levels; however, no measurements of CA or Rubisco activity were made in that study (Raines et al., 1992). In contrast to higher plant studies, the response of CA expression during acclimation to limiting levels of C₃ is well documented in eukaryotic algae (Coleman, 1991; Badger and Price, 1992; Sultemeyer et al., 1993). Extracellular levels of C₃ appear to regulate transcriptional activity of genes encoding various isoforms of CA, and transfer of cells from one CO₂ level to another results in rapid modification of ca mRNA abundance, protein level, and activity (Bailey and Coleman, 1988; Fukuzawa et al., 1990).

In this study we examined CA and Rubisco expression in pea (Pisum sativum) leaves acclimated to ambient, elevated, and below-ambient levels of CO₂. In addition, we have examined the capacity of the plants to modify CA and Rubisco activity and transcript abundance in response to rapid changes in CO₂ levels. The levels of soluble foliar carbohydrates translocated from leaves into sink tissues were also measured. The results of these studies provide insights into the regulation of CA expression in response to changes in CO₂ concentration.
carbohydrates were also determined in an effort to correlate changes in gene expression with leaf carbohydrate abundance.

**MATERIALS AND METHODS**

Seeds of pea (*Pisum sativum* cv Little Marvel) were grown in growth chambers at the appropriate CO₂ concentration for 3 weeks in complete soil, with a daylength of 16 h, PPFD of 400 μmol m⁻² s⁻¹, and a day/night temperature regime of 20/16°C. Plants were watered daily and fertilized weekly with a commercial preparation (PlantProd 20–20–20 with micronutrients, Plant Products, Brampton, Ontario, Canada). Pots were 20 cm in diameter with no more than three plants per pot. When required, plants were transferred to different CO₂ concentrations following 2.5 weeks of growth at high (1000 μL/L), ambient (350 μL/L), or low-CO₂ (160 μL/L) concentrations. Analyses were performed using the first set of fully expanded (mature) leaves or the set of developing (immature) leaves proximal to the apex.

**Enzyme Activities**

Leaves were excised from fully illuminated plants, their areas and fresh weights quickly determined, and then they were frozen in liquid nitrogen. When required, these frozen samples were ground with a mortar and pestle in extraction buffer (100 mM Bicine, pH 8.2, 20 mM MgCl₂, 5 mM DTT, 1 mM EDTA), and the samples were clarified by centrifugation for 10 min at 10,000g at 4°C. Total CA activity was determined electrometrically (Wilbur and Anderson, 1948). The rate of RuBP-dependent ¹⁴CO₂ incorporation at 25°C following full CO₂/Mg²⁺ activation of the enzyme was used to determine Rubisco activity (Hudson et al., 1992). Enzymes were assayed in triplicate and expressed on the basis of leaf area. Supernatant fractions were assayed for soluble protein (Bradford, 1976), and chlorophyll concentrations were determined (Porra et al., 1989).

**Foliar Soluble Sugar Assays**

Leaves from illuminated plants growing at the appropriate CO₂ concentrations were excised, their fresh weights and areas quickly determined, and then they were frozen in liquid nitrogen and ground in a mortar and pestle prior to perchloric acid extraction as described by Stitt et al. (1989). Foliar levels of Glc, Fru, and Suc were assayed using a coupled-enzyme system described previously (Stitt et al., 1989).

**RNA Isolation and Northern Blot Hybridization**

Isolation of total RNA from tissue harvested at the appropriate time was achieved using a previously described protocol (Majeau and Coleman, 1994). Equal aliquots of RNA (5 μg in each sample as determined spectrophotometrically) were denatured with formaldehyde:formamide (6:5:50%, v/v) and then immobilized on nitrocellulose using a slot-blot apparatus (Minifold II Slot-Blotter, Schleicher & Schuell) (Fourney et al., 1988). Prehybridization, hybridization, and probe-labeling protocols were all as described previously (Majeau and Coleman, 1994). Gene probes used were a 0.95-kb pea chloroplast *ca* cDNA (Majeau and Coleman, 1991), a 0.68-kb pea *rbcS* cDNA (Coruzzi et al., 1983), and an 18S soybean rDNA sequence used for normalization of RNA loads. Quantification of hybridization to pea RNA by all probes was achieved by phosphorimaging of the blots (model 400S, Molecular Dynamics, Sunnyvale, CA).

**RESULTS**

Growth of pea plants at different CO₂ concentrations resulted in major changes in leaf area and fresh weight, Rubisco and CA activity, and soluble protein content (Table I). High CO₂ levels (1000 μL/L) increased individual leaf area, as well as leaf fresh weight. In contrast, plants grown under low-CO₂ conditions (160 μL/L) had smaller leaves (both area and fresh weight) than control plants. In vitro Rubisco activity in the youngest, fully expanded leaves was measured following full activation of the enzyme. A greater than 35% reduction in activity (expressed on a leaf area basis) was found in leaves grown at high levels of CO₂ compared with air-grown plants. Growth of plants at low-CO₂ concentrations resulted in no significant decline in total activity. CA activity in high-CO₂-grown plants was reduced by 30%, whereas low-CO₂-grown plants exhibited an increase in CA activity of approximately 50% relative to air-grown plants.

To investigate the effects of different CO₂ growth conditions on *ca* and *rbcS* transcript abundance, total RNA was extracted from mature leaves (pairs of youngest, fully expanded leaves, as described in "Materials and Methods") of plants germinated and grown for 3 weeks at 160, 350, and 1000 μL/L CO₂. The results of the slot-blot analysis are shown in Figure 1. *ca* transcript levels for plants grown at high-CO₂ were reduced to less than 30% of that observed in plants grown in air; *rbcS* transcript levels were reduced in a similar fashion. Growth at 160 μL/L CO₂ also resulted in a decline in *ca* and *rbcS* transcript abundance below that

| CO₂ Concentration | Leaf fresh weight (mg) | Leaf area (cm²) | Chlorophyll (mg m⁻²) | Soluble protein (mg m⁻²) | CA activity (units m⁻² 10⁻⁹) | Rubisco activity (μmol s⁻¹ m⁻²) |
|-------------------|------------------------|----------------|---------------------|-------------------------|----------------------------|-------------------------------|
| High (1000 μL/L)  | 98 ± 11                | 5.8 ± 0.8      | 267 ± 23            | 2.05 ± 0.13             | 7.87 ± 1.18                | 33.2 ± 5.9                   |
| Ambient (350 μL/L)| 55 ± 14                | 4.3 ± 1.2      | 251 ± 27            | 2.47 ± 0.44             | 11.39 ± 0.97               | 51.2 ± 5.5                   |
| Low (160 μL/L)    | 20 ± 7                 | 1.2 ± 0.2      | 291 ± 30            | 3.09 ± 0.37             | 17.43 ± 1.22               | 49.1 ± 6.8                   |

Values represent means ± se of a minimum of six plant replicates from two separate experiments.
exhibited by the air-grown control plants, although the reduction in \( \text{ca} \) levels was less pronounced than that observed under high-CO\(_2\) conditions (Fig. 1).

The effect on CA and Rubisco expression following transfer of pea plants from one CO\(_2\) concentration to another was also examined. Plants grown for 2.5 weeks at high levels of CO\(_2\) were transferred to ambient conditions, and their \( \text{ca} \) and \( \text{rbcS} \) transcript abundance in mature leaves determined (Fig. 2a). Both \( \text{ca} \) and \( \text{rbcS} \) transcript abundance exhibited a rapid increase following exposure to air, reaching maximum levels after 3 h. This was followed by a gradual decline, during which mRNA levels returned to the levels found in air-grown plants. Northern blot analysis of RNA isolated from immature leaves showed no changes in transcript abundance following transfer from high to air levels of CO\(_2\) (data not shown); the effect on transcript abundance following transfer from air to high levels of CO\(_2\) is shown in Figure 2b. Although the changes are less pronounced there appeared to be a decline in \( \text{ca} \) transcript abundance, with the lowest levels achieved at the 3-h time point, followed by a gradual increase to levels somewhat higher than those seen in high-CO\(_2\)-grown plants. Changes in \( \text{rbcS} \) transcript levels exhibited no discernible pattern, although the lowest value was obtained at the 3-h time point. Even after 12 h of exposure to 1000 \( \mu \text{L/L} \) CO\(_2\), \( \text{ca} \) and \( \text{rbcS} \) transcript levels were still somewhat higher than those observed in high-CO\(_2\)-grown plants.

CA and Rubisco activities were also examined in mature leaves acclimated for 24 h following transfer to a different CO\(_2\) concentration (Table II). Exposure of high-CO\(_2\)-grown plants to a lower CO\(_2\) concentration resulted in an increase in the activity of both enzymes, with levels approaching those observed in air-grown plants.

![Figure 1](image1.png)

**Figure 1.** \( \text{ca} \) and \( \text{rbcS} \) transcript abundance of mature leaves grown at high (1000 \( \mu \text{L/L} \)), ambient (350 \( \mu \text{L/L} \)), and low (160 \( \mu \text{L/L} \)) concentrations of CO\(_2\). Slot blots (5 \( \mu \text{g} \) of total RNA per slot) were probed with \(^{32}\text{P}\)dCTP-labeled cDNA encoding pea \( \text{ca} \) and \( \text{rbcS} \). A radiolabeled soybean rDNA probe was used to ensure that each slot contained equal aliquots of pea RNA. The extent of probe hybridization (expressed as counts detected by phosphorimaging) to RNA in pooled extracts obtained from 15 individual plants and represent the means ± SE of three sample replicates.

![Figure 2](image2.png)

**Figure 2.** Time course of change in \( \text{ca} \) and \( \text{rbcS} \) transcript abundance following transfer of plants from 1000 to 350 \( \mu \text{L/L} \) CO\(_2\) (a), and from 350 to 1000 \( \mu \text{L/L} \) CO\(_2\) (b). Each slot contains 5 \( \mu \text{g} \) of total RNA and was probed with labeled cDNA encoding pea \( \text{ca} \) and \( \text{rbcS} \), as well as with a soybean rDNA sequence for normalization of RNA loading. Data show the extent of probe hybridization (expressed as counts detected by phosphorimaging) to RNA in pooled extracts obtained from 15 individual plants and represent the means ± SE of three sample replicates.

**Table II.** CA and Rubisco activities after transfer from high to ambient or low-CO\(_2\) concentrations

| Characteristics       | CO\(_2\) Concentration |
|-----------------------|------------------------|
|                       | High (1000 \( \mu \text{L/L} \)) | Ambient (350 \( \mu \text{L/L} \)) | Low (160 \( \mu \text{L/L} \)) |
| CA activity (units \( \text{m}^{-2} \text{s}^{-1} \)) | 7.17 ± 0.45 | 9.49 ± 1.79 | 10.65 ± 0.74 |
| Rubisco activity (\( \text{fimol s}^{-1} \text{m}^{-2} \)) | 34.9 ± 4.6 | 42.6 ± 5.7 | 41.3 ± 6.4 |

The youngest, fully expanded leaves were analyzed after 24 h of exposure to a lower CO\(_2\) concentration. Values represent means ± se of a minimum of four plant replicates from two experiments.
plants to high-\(\text{CO}_2\) concentrations for 24 h did not change CA or Rubisco activities in mature leaves (data not shown).

Levels of Fru/Glc and Suc were determined in the youngest, fully expanded leaf pairs obtained from plants grown at or transferred to various \(\text{CO}_2\) concentrations (Table III). As expected, levels of Fru/Glc and Suc were highest in plants grown at 1000 \(\mu\text{L/L}\) \(\text{CO}_2\). Transfer from high to air levels of \(\text{CO}_2\) resulted in a significant decline in foliar Suc. Leaves from plants grown at air levels of \(\text{CO}_2\) contained lower amounts of Fru/Glc and Suc. Suc levels increased rapidly following transfer from air to high levels of \(\text{CO}_2\).

## DISCUSSION

In this study we examined the effect of long- and short-term exposure to varying \(\text{CO}_2\) concentrations on the expression of CA and Rubisco in pea leaves. Transcript abundance and enzymatic activity of both proteins decreased in mature leaves when plants were grown at a \(\text{CO}_2\) concentration of 1000 \(\mu\text{L/L}\). As recently reviewed by Bowes (1991) and Webber et al. (1994), there are many examples of exposure to high-\(\text{CO}_2\) concentrations lowering both CA and Rubisco activities in \(\text{C}_3\) plants. Lowering of Rubisco activity in response to elevated \(\text{CO}_2\) has been ascribed to enzyme inactivation, inhibition of translation, and modulation of transcript abundance. In this study we show that down-regulation of both Rubisco and CA activity is accompanied by a reduction in \(\text{rbcS}\) and \(\text{ca}\) transcript levels. Recent studies have also shown that growth at high-\(\text{CO}_2\) concentrations results in a decline in Rubisco activity (Xu et al., 1994) and transcript abundance (Riviere-Rolland et al., 1996) in pea. The latter study also indicated that high-\(\text{CO}_2\) repression of Rubisco expression occurred only during growth at limiting N conditions. Although the N status of plants was not monitored during our study, the fertilization regime should have provided sufficient N, and no symptoms of N deficiency were observed. Cultivar variation in N utilization or allocation may account for the differences between the two studies.

In contrast to pea, in the only other published study (to our knowledge) on the \(\text{CO}_2\) regulation of \(\text{ca}\) gene expression, increased \(\text{ca}\) transcript abundance was observed when \(\text{A. thaliana}\) plants were grown at 660 \(\mu\text{L/L}\) \(\text{CO}_2\) (Raines et al., 1992). No measurements of activity or protein levels were reported, however, and it is not known if increased transcript levels resulted in changes in activity. The results from Arabidopsis may indicate that the control of CA expression by \(\text{CO}_2\) concentration could vary between species, or that the lower \(\text{CO}_2\) concentration used in the Arabidopsis study promotes CA expression. It has been reported that cotton grown at 660 \(\mu\text{L/L}\) \(\text{CO}_2\) exhibits increased CA activity compared with air-grown plants, but growth at 1000 \(\mu\text{L/L}\) \(\text{CO}_2\) represses CA activity (Chang, 1975). Plants grown at \(\text{CO}_2\) concentrations below ambient also display a reduction in Rubisco activity, but have elevated levels of CA activity compared with air-grown plants. The decline in Rubisco activity, as well as modification of other leaf characteristics, are similar to those observed in the \(\text{C}_3\) plant \(\text{Abutilon theophrasti}\) grown at 150 \(\mu\text{L/L}\) \(\text{CO}_2\) (Tissue et al., 1994). Although CA levels were not measured in the \(\text{Abutilon}\) study, chlorophyll, Rubisco content and activity, and leaf mass were all reduced in plants grown at 150 \(\mu\text{L/L}\) compared with air-grown plants. In pea, \(\text{ca}\) transcript levels in mature leaves do not reflect enhanced enzyme activity, but are higher than those found in high-\(\text{CO}_2\)-grown plants. The difference between \(\text{ca}\) transcript abundance and activity may be a function of enhanced translational activity and/or a reduced rate of CA turnover under these growth conditions; these possibilities are under further study. Rubisco activity more closely parallels transcript abundance. Increased CA activity (which results in the CA/Rubisco ratio being enhanced) in plants grown at low-\(\text{CO}_2\) levels may assist in the diffusion of \(\text{C}_4\) through the chloroplast and with \(\text{HCO}_3^-\) hydration at the site of fixation.

Both \(\text{ca}\) and \(\text{rbcS}\) transcript levels of high-\(\text{CO}_2\)-grown plants increased significantly after a 3-h exposure to air levels of \(\text{CO}_2\). There was also a significant increase in both CA and Rubisco activity 24 h after transfer from high-\(\text{CO}_2\) to ambient or below-ambient levels of \(\text{CO}_2\). It has recently been suggested that the modulation of nuclear-encoded photosynthetic gene expression by \(\text{CO}_2\) is mediated by changes in the soluble carbohydrate concentration of the leaf (Stitt, 1991; van Oosten et al., 1994; van Oosten and Besford, 1995). High external \(\text{CO}_2\) concentration results in the accumulation of elevated foliar levels of starch and soluble sugars when the rate of photosynthetic carbon fixation exceeds the translocation rate to sink tissues. Elevated levels of foliar carbohydrates are associated with reduced abundance of a number of transcripts encoding proteins required for carbon fixation (van Oosten et al., 1994). Similar results have been obtained by supplying

### Table III. Soluble carbohydrate content in leaves exposed to high or ambient levels of \(\text{CO}_2\)

| Carbohydrate Concentration (mmol m\(^{-2}\)) | \(\text{CO}_2\) Concentration | Glc and Fru | Suc |
|---------------------------------------------|-----------------------------|------------|-----|
| High\(^a\) (1000 \(\mu\text{L/L}\))          | Ambient\(^a\) (350 \(\mu\text{L/L}\)) | 6.6 ± 1.8  | 1.7 ± 0.3 |
|                                              | High to ambient\(^b\) (3 h) | 5.6 ± 1.3  | 4.1 ± 1.1 |
|                                              | (24 h) | 6.4 ± 0.9  | 2.8 ± 0.4 |
|                                              | Ambient to high\(^b\) (3 h) | 10.7 ± 1.3 | 3.0 ± 0.5 |
|                                              | (24 h) | 9.7 ± 3.1  | 7.5 ± 1.0 |

\(^a\) Grown continuously at the indicated \(\text{CO}_2\) concentration. \(^b\) Transferred from growth \(\text{CO}_2\) concentration to indicated \(\text{CO}_2\) concentration and carbohydrate levels assayed after 3 or 24 h.
detached leaves with external sources of hexoses (Krapp et al., 1991, 1993; van Oosten et al., 1994). Convincing evidence for specific and coordinated hexose-dependent repression of transcription was provided in studies using reporter sequences fused to promoter regions obtained from maize photosynthetic genes (Sheen, 1990, 1994). In our study, the transfer of plants from high to air levels of CO₂ rapidly reduced the foliar levels of carbohydrates by decreasing photosynthesis but permitting continued translocation. The rapid increase in ca and rbcS mRNA levels could be a response to the removal of carbohydrate repression of transcriptional activity. The changes in ca and rbcS transcript abundance result in increases in enzyme activity following a 24-h exposure to air levels of CO₂.

The transfer of plants from air to high levels of CO₂ did not result in definitive changes in transcript abundance within the 12-h time course of the experiments. In our studies, it was only after long-term exposure to high levels of CO₂ that decreased CA and Rubisco expression was observed.

Immature pea leaves failed to down-regulate ca and rbcS transcript levels in response to elevated CO₂ concentrations. This observation is in agreement with the carbohydrate model of transcriptional regulation, as these immature tissues are effective sinks for carbohydrate reserves. In addition, the developmental stage of the nascent leaf may preclude modulation of gene expression by carbohydrate levels. In a recent publication by Nie et al. (1995), high-CO₂ (550 µL/L) field-grown wheat exhibited a similar down-regulation of a number of nuclear-encoded photosynthetic genes, but CO₂ modulation of gene expression was highly dependent on the stage of leaf and crop development. These data show that a number of additional factors, both environmental and developmental, may override carbohydrate regulation of gene expression in the field.

In our studies with pea, if we assume that elevated levels of foliar carbohydrates were responsible for the down-regulation of CA and Rubisco expression in high-CO₂-grown plants, this suggests that the source-to-sink allocation of photosynthetic assimilates was limited during these experiments. Although we attempted to maximize growth space (few plants per pot) and maintained the plants in a well-watered and fertilized environment, it is still possible that resource allocation to sinks such as roots was restricted by the physical environment. Further studies will be required to determine if pea translocation capacity is limited by biotic or abiotic factors when plants are grown at high-CO₂ concentrations.

In conclusion, the data presented in this paper are generally consistent with the proposed model for carbohydrate regulation of photosynthetic gene expression in higher plants. It is also interesting to note that CA expression responds to below-ambient levels of CO₂ in a manner somewhat analogous to that observed in eukaryotic algae. The ability of the plant to rapidly modulate ca and rbcS transcript abundance in response to external CO₂ concentrations suggests that regulation of gene expression may be an important general mechanism in the acclimation of photosynthesis to changing environmental parameters.

Received March 12, 1996; accepted July 5, 1996.
Copyright Clearance Center: 0032-0889/96/112/0569/06.

LITERATURE CITED

Badger MR, Price GD (1992) The CO₂ concentrating mechanism in cyanobacteria and green algae. Physiol Plant 84: 606–615
Badger MR, Price GD (1994) The role of carbonic anhydrase in photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 45: 369–392
Bailly J, Coleman JR (1988) Effect of CO₂ concentration on protein biosynthesis and carbonic anhydrase expression in Chlamydomonas reinhardtii. Plant Physiol 87: 833–840
Besford RT, Ludwig LJ, Withers AC (1990) The greenhouse effect: acclimation of tomato plants growing in high-CO₂, photosynthesis and ribulose-1,5-bisphosphate carboxylase protein. J Exp Bot 41: 925–931
Bowers G (1991) Growth at elevated CO₂: photosynthetic responses mediated through Rubisco. Plant Cell Environ 14: 795–806
Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254
Cervigni T, Teofani F, Bassanelli C (1971) Effect of CO₂ on carbonic anhydrase in Avena sativa and Zea mays. Phytochemistry 10: 2291–2294
Chang CW (1975) Carbon dioxide and senescence in cotton plants. Plant Physiol 55: 515–519
Coleman JR (1991) The molecular and biochemical analyses of CO₂ concentrating mechanisms in cyanobacteria and green algae. Plant Cell Environ 14: 861–867
Coxon P, Brogle R, Cashmore A, Chua N-H (1983) Nucleotide sequences of two cDNA clones encoding the small subunit of ribulose-1,5-bisphosphate carboxylase and the major chlorophyll a/b binding thylakoid polypeptide. J Biol Chem 258: 1399–1402
Fourney RM, Miyakoshi J, Day RS III, Patterson MC (1988) Northern blotting: efficient RNA staining and transfer. BRL Focus 10: 5–7
Fukuzawa H, Fujiwara S, Yamamoto Y, Dionisio-Sese ML, Miyachi S (1990) cDNA cloning, sequence, and expression of carbonic anhydrase in Chlamydomonas reinhardtii: regulation by environmental CO₂ concentration. Proc Natl Acad Sci USA 87: 4383–4387
Hudson GS, Evans JR, von Caemmerer S, Arvidson YBC, Andrews TJ (1992) Reduction of ribulose-1,5-bisphosphate carboxylase/oxygenase content by antisense RNA reduces photosynthesis in transgenic tobacco. Plant Physiol 98: 294–302
Krapp A, Hofmann B, Schafer C, Stitt M (1993) Regulation of the expression of rbcS and other photosynthetic genes by carbohydrates: a mechanism for the "sink regulation" of photosynthesis? Plant J 3: 817–828
Krapp A, Quick P, Stitt M (1991) Ribulose-1,5-bisphosphate carboxylase/oxygenase, other Calvin-cycle enzymes, and chlorophyll decrease when glucose is supplied to mature spinach leaves via the transpiration stream. Planta 186: 58–69
Majeau N, Arnoldo MA, Coleman JR (1994) Modification of carbonic anhydrase activity by antisense and over-expression constructs in transgenic tobacco. Plant Mol Biol 25: 377–385
Majeau N, Coleman JR (1991) Isolation and characterization of a cDNA coding for pea chloroplastic carbonic anhydrase. Plant Physiol 95: 264-268
Majeau N, Coleman JR (1994) Correlation of carbonic anhydrase and ribulose-1,5-bisphosphate carboxylase/oxygenase expression in pea. Plant Physiol 104: 1393-1399
Nie G, Hendrix DL, Webber AN, Kimball BA, Long SP (1995) Increased accumulation of carbohydrates and decreased photosynthetic gene transcript levels in wheat grown at an elevated CO2 concentration in the field. Plant Physiol 108: 975-983
Peet MM, Huber SC, Patterson DT (1986) Acclimation to high-CO2 in monocotyledonous cucumbers. II. Carbon exchange rates, enzyme activities, and starch and nutrient concentrations. Plant Physiol 86: 63-67
Porra RJ, Thompson WA, Kreidemann PE (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophyll a and b extracted with four solvents: verification of the concentration of chlorophyll standards by absorption spectroscopy. Biochim Biophys Acta 975: 384-394
Porter MA, Grodzinski B (1984) Acclimation to CO2 in bean. Carbonic anhydrase and ribulose bisphosphate carboxylase. Plant Physiol 74: 413-416
Price GD, von Caemmerer S, Yu J-W, Lloyd J, Oja V, Kell P, Harrison K, Gallagher A, Badger MR (1994) Specific reduction of the chloroplast carbonic anhydrase activity by antisense RNA in transgenic tobacco has a minor effect on photosynthetic CO2 assimilation. Planta 193: 331-340
Raines CA, Horsnell PR, Holder C, Lloyd JC (1992) Arabidopsis thaliana carbonic anhydrase: cDNA sequence and effect of CO2 on mRNA levels. Plant Mol Biol 19: 113-114
Riviere-Rolland H, Contard P, Betsche T (1996) Adaptation of pea to elevated atmospheric CO2: Rubisco, phosphoenolpyruvate carboxylase and chloroplast phosphate translocator at different levels of nitrogen and phosphorus nutrition. Plant Cell Environ 19: 109-117
Rowland-Bamford AJ, Baker JT, Allen LH, Bowes G (1991) Acclimation of rice to changing atmospheric carbon dioxide concentration. Plant Cell Environ 14: 577-583
Sage RF (1994) Acclimation of photosynthesis to increasing atmospheric CO2: the gas exchange perspective. Photosynth Res 39: 351-368
Sage RF, Sharkey TD, Seemann JR (1989) Acclimation of photosynthesis to elevated CO2 in five C3 species. Plant Physiol 90: 590-596
Sheen J (1990) Metabolic repression of transcription in higher plants. Plant Cell 2: 1027-1038
Sheen J (1994) Feedback control of gene expression. Photosynth Res 39: 427-438
Stitt M (1991) Rising CO2 levels and their potential significance for carbon flow in photosynthetic cells. Plant Cell Environ 14: 741-762
Stitt M, Lilley R, Gerhardt R, Heldt HW (1989) Determination of metabolite levels in specific cells and subcellular compartments of plant leaves. Methods Enzymol 174: 518-532
Sultemeyer D, Schmidt C, Fock HP (1993) Carbonic anhydrases in higher plants and aquatic microorganisms. Physiol Plant 88: 179-190
Tissue DT, Griffin KL, Thomas RB, Strain BR (1994) Effects of low and elevated CO2 on C4 and C3 annuals. II. Photosynthesis and biochemistry. Oecologia 101: 21-28
van Oosten JJ, Besford RT (1995) Some relationships between the gas exchange, biochemistry and molecular biology of photosynthesis during leaf development of tomato plants after transfer to different carbon dioxide concentrations. Plant Cell Environ 18: 1253-1266
van Oosten JJ, Wilkins D, Besford RT (1994) Regulation of the expression of photosynthetic nuclear genes by CO2 is mimicked by regulation by carbohydrates: a mechanism for the acclimation of photosynthesis to high-CO2? Plant Cell Environ 17: 913-923
Webber AN, Nie GY, Long SP (1994) Acclimation of photosynthetic proteins to rising atmospheric CO2. Photosynth Res 39: 413-425
Wilbur KM, Anderson NG (1948) Electrometric and colorimetric determination of carbonic anhydrase. J Biol Chem 176: 147-154
Xu D-Q, Gifford RM, Chow WS (1994) Photosynthetic acclimation in pea and soybeans to high atmospheric CO2 partial pressure. Plant Physiol 106: 661-671