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Mini Review

Photosynthesis and Plant Growth at Elevated Levels of CO_2

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In this review, we discuss the effects of elevated CO_2 levels on photosynthesis in relation to the whole plant growth in terrestrial higher C_3 plants. Short-term CO_2 enrichment stimulates the rate of photosynthesis. Plant mass is also enhanced by CO_2 enrichment. However, the effects of long-term CO_2 enrichment on photosynthesis are variable. Generally, the prolonged exposure to CO_2 enrichment reduces the initial stimulation of photosynthesis in many species, and frequently suppresses photosynthesis. These responses are attributed to secondary responses related to either excess carbohydrate accumulation or decreased N content rather than direct responses to CO_2. Accumulation of carbohydrates in leaves may lead to the repression of photosynthetic gene expression and excess starch seems to hinder CO_2 diffusion. Therefore, the species which have the sink organs for carbohydrate accumulation do not show the suppression of photosynthesis. The suppression of photosynthesis by CO_2 enrichment is always associated with decreases in leaf N and Rubisco contents. These decreases are not due to dilution of N caused by a relative increase in the plant mass but are the result of a decrease in N allocation to leaves at the level of the whole plant, and the decrease in Rubisco content is not selective. Leaf senescence and plant development are also accelerated by CO_2 enrichment. However, they are independent of each other in some species. Thus, various responses to CO_2 observed at the level of a single leaf result from manifold responses at the level of the whole plant grown under conditions of CO_2 enrichment.

Key words: C_3 plants — Carbohydrate — CO_2 enrichment — Nitrogen — Ribulose-1,5-bisphosphate carboxylase/oxygenase.

Elevated atmospheric CO_2 levels strongly affect photosynthesis and growth of many plants, especially C_3 plants which constitute more than 90% of terrestrial species. The CO_2 level in atmosphere was stabilized at about 28 Pa (280 ppm) for these 100,000 years, but it has been rising after the Industrial Revolution and the present level is about 36 Pa. Current predictions are that atmospheric CO_2 levels will double during the next century. For plants adapted originally to a preindustrial CO_2 level of 28 Pa, this is a substantial increase in CO_2 supply. Generally, CO_2 enrichment stimulates the rate of photosynthesis and growth. Plant mass is also enhanced at elevated levels of CO_2. However, long-term CO_2 enrichment during a period of weeks to months reduces the initial stimulation of photosynthesis and then frequently suppresses photosynthesis. These findings indicate that the effects of CO_2 enrichment on photosynthesis are very complicated, and we must distinguish between the short-term and long-term effects of CO_2.

In this review, we discuss the effect of elevated levels of CO_2 on photosynthesis in relation to the whole-plant growth in terrestrial higher C_3 plants. We first describe the short-term response of photosynthesis to elevated CO_2 and then consider the long-term effects of CO_2 enrichment at the biochemical and physiological levels. In addition, we discuss such short- and long-term responses of photosynthesis in relation to the differences in the growth strategies at the level of the whole plant depending on the species, the developmental stage, and the developmental conditions. A few reviews dealing with biochemical and physiological aspects of photosynthesis and plant growth at elevated levels of CO_2 have been published (Stitt 1991, Drake et al. 1997, Moore et al. 1999, Stitt and Krapp 1999).

Short-term response of photosynthesis to CO_2 enrichment

In this section, we describe the effect of the short-term CO_2 enrichment during a period of seconds to hours on the rate of photosynthesis. We first analyze the CO_2 response of photosynthesis from the Rubisco kinetics, and then consider how the increased photosynthesis affects the various reactions of photosynthesis.

CO_2 diffusion—CO_2 flows from the atmosphere to intercellular air spaces through the stomatal pore, and diffuses across the wall, plasmalemma, cytosol and the chloroplast envelope, and to the stroma. The partial pressure of CO_2 in the intercellular air spaces (*pCi*) is controlled by stomatal opening. Generally, as ambient CO_2 partial pressures (*pCa*) increase, stomata tend to close (for rev-

Abbreviations: *pCa*, ambient CO_2 partial pressure; *pCc*, chloroplastic CO_2 partial pressure; *pCi*, intercellular CO_2 partial pressure; RuBP, ribulose 1,5-bisphosphate; Rubisco, RuBP carboxylase/oxygenase.

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iews, see Assmann 1999, Jarvis et al. 1999). A mechanism(s) controlling CO_2 diffusion from the intercellular air spaces to the chloroplast stroma has not been identified, but this seems be related to the chloroplast movement (Terashima, personal communication). The chloroplast movement may occur by actin filaments (Kadota and Wada 1992, Haupt and Scheuerlein 1990). In leaves with high photosynthetic capacity, chloroplasts avoid the membranes of the cells attached to the neighbouring cells and adhere effectively to the membranes which are exposed to air spaces (Terashima et al. 1995, Evans and von Caemmerer 1996). Probably, such a chloroplast movement within the cells may also regulate the partial pressure of CO_2 in the chloroplast stroma (*pCc*).

CO_2 and Rubisco kinetics—The fixation of CO_2 into photosynthetic metabolism is catalyzed by ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (Rubisco, for a review, see Lorimer 1981). This enzyme also catalyzes competitively the production of 2-phosphoglycolate in the photorespiratory pathway with O_2 as another substrate. The ratio of these two reactions by Rubisco (carboxylation and oxygenation of RuBP) depends on the ratio of the partial pressure of CO_2 to that of O_2. Since the respective $K_m(CO_2)$ and $K_m(O_2)$ values at 25°C of Rubisco from higher plants are close to the partial pressures of CO_2 and O_2 in the atmospheric air, elevating CO_2 levels in air stimulate the carboxylation and suppress the oxygenation. However, the increase in the photosynthetic rate observed above the present atmospheric CO_2 level is generally smaller than that predicted from the Rubisco kinetics. For example, whereas an elevation of *pCa* from 36 Pa to 72 Pa (20 Pa to 50 Pa at *pCc*) at 25°C enhances the carboxylation rate by about two times according to the Rubisco kinetics, the actual increase of photosynthesis observed lies in the range of 25 to 60%. This means that photosynthesis under conditions of CO_2 enrichment is limited by other components limiting photosynthesis.

CO_2 and limitation of photosynthesis—The response of C_3-photosynthesis to CO_2 was theoretically modelled by Farquhar's group (Farquhar et al. 1980, Farquhar and von Caemmerer 1982). According to their model, the photosynthetic rate is limited by either Rubisco capacity or the capacity of the thylakoid reactions to regenerate RuBP. In their model, the rate of electron transport reflects the capacity of RuBP regeneration. Sharkey (1985a) modified this model and added a limitation by the availability of P_i in the chloroplast for ATP synthesis to RuBP-regeneration limitation. Their model is summarized in Fig. 1, using our data published before (Makino et al. 1994a). Rubisco capacity limits photosynthesis at the present *pCa* and below, under saturating light conditions (von Caemmerer and Farquhar 1981, Makino et al. 1985, Evans 1986). Above the present *pCa*, electron-transport capacity limits photosynthesis (von Caemmerer and Farquhar 1981, Evans and

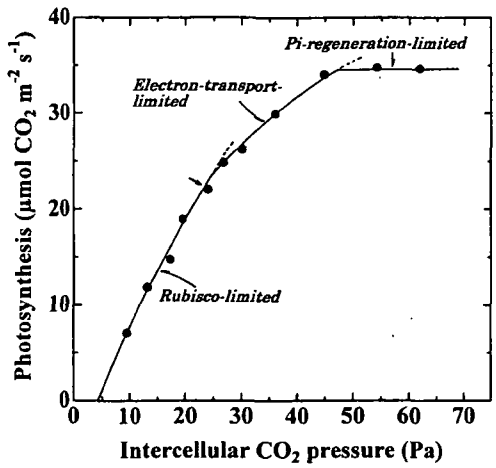

Fig. 1 Modelled photosynthetic rate as a function of *pCi* in the leaf of C_3 species. Experimental data are taken from figure 2 in Makino et al. (1994a) with rice. The arrow indicates the point obtained at a *pCa* of 36 Pa. Measurements were made at a leaf temperature of 25°C, an irradiance of 1,800 μmol quanta m^{-2} s^{-1}, and a leaf-to-air vapor pressure difference of 1.0 to 1.2 kPa. Three model curves are fitted to the data; Rubisco-limited (equation 16.61 in Farquhar and von Caemmerer 1982), Electron-transport-limited (equation 16.62 in Farquhar and von Caemmerer 1982), and P_i-regeneration-limited (equation 16 in Sharkey 1985a).

Terashima 1988). In addition, at a higher *pCi*, the availability of P_i also contributes to the limitation (Sharkey 1985b, Stitt 1986, Sivak and Walker 1986, Sage and Sharkey 1987, Stitt and Quick 1989). This P_i-regeneration limitation reflects the rate at which the intermediate products of CO_2 fixation (triose-phosphate) are converted to starch and sucrose; the availability of P_i is determined by the capacity of starch and sucrose synthesis to regenerate P_i from phosphorylated intermediates. Therefore, this limitation has been termed triose-phosphate-utilization limitation (Sharkey 1985a), P_i limitation (Sivak and Walker 1986), P_i-regeneration limitation (Sage et al. 1988) or end-product synthesis limitation (Stitt 1991). Thus, photosynthesis at elevated CO_2 levels is limited by either electron-transport capacity or P_i-regeneration capacity during starch and sucrose synthesis. Both limitations eventually determine RuBP regeneration for Rubisco activity. However, these can be distinguished. Whereas the P_i-regeneration-limited photosynthesis is unaffected by elevating *pCi*, photosynthesis limited by the electron-transport capacity is still increased by elevating *pCi*. Actually, the electron transport capacity is also independent of *pCi*, but the increase in photosynthesis at *pCi* where electron-transport becomes limiting results only from an increase in carboxylation relative to oxygenation for a given rate of RuBP regeneration.

It has been frequently reported that although Rubisco is fully activated around the normal *pCa* where Rubisco

is limiting, CO_2 enrichment substantially decreases the activation state of Rubisco in several C_3 species such as *Raphanus sativus* (von Caemmerer and Edmondson 1986), bean (Sage et al. 1988, Socias et al. 1993), lamb's-quarters (Sage et al. 1990), cabbage and eggplant (Sage et al. 1989) and wheat (Theobald et al. 1998). This deactivation of Rubisco has been interpreted as a secondary response to the maintenance of the balance between Rubisco and other processes limiting photosynthesis at elevated *pCa* (Sage et al. 1988, Sage 1990, Stitt 1991). On the other hand, there have been some reports that Rubisco remains fully activated even at elevated *pCa* in soybean (Campbell et al. 1988, Sicher et al. 1995, Vu et al. 1997), loblolly pine (Tissue et al. 1993), tobacco (Sicher and Kremer 1994), pea (Xu et al. 1994) and rice (Rowland-Bamford et al. 1991, Nakano et al. 1997, Vu et al. 1997). However, this does not mean that photosynthesis in these species is limited by Rubisco capacity over a wide range of *pCi*. For example, in rice, the maximum Rubisco activity estimated at $pCa = 100$ Pa from the Rubisco kinetics is 1.5- to 2-fold greater than the actual photosynthetic rate observed at the same *pCa* (Makino et al. 1997c, also see Fig. 1). This indicates that Rubisco efficiency for CO_2-saturated photosynthesis is 50 to 70%, and this is close to the activation ratio of Rubisco observed at elevated levels of CO_2 in bean by Sage et al. (1988). Thus, under conditions of CO_2 enrichment, photosynthesis is limited by either electron transport capacity or P_i-regeneration capacity, and Rubisco is excessive irrespective of change in the activation state.

Long-term response of photosynthesis to CO_2 enrichment

Many studies have shown that CO_2 enrichment stimulates photosynthesis and enhances plant mass. However, the initial stimulation of net photosynthesis by CO_2 enrichment decreases during the subsequent exposure to elevated CO_2, and then a suppression of photosynthesis often occurs (for a review, see Stitt 1991). This means that prolonged exposure to elevated CO_2 leads to changes in biochemical, physiological or morphological factors which remove or offset the initial stimulation of photosynthesis. On the other hand, there are some species showing little down-regulation of photosynthesis even when they are grown during long-term CO_2 enrichment. Thus, it is difficult to generalize the long-term response to elevated CO_2. In this section, we describe possible factors underlying the suppression of photosynthesis under long-term CO_2 enrichment during a period of weeks to months, and then consider the biochemical mechanisms which are involved in the down-regulation of photosynthesis at the level of a single leaf.

Carbohydrate accumulation—Accumulation of carbohydrates has been observed in many studies on plant growth under CO_2 enrichment. This is because the photosynthetic rate exceeds the sink capacity to utilize the photosynthate for growth. Therefore, much attention has been paid to whether there is a causal relationship(s) between carbohydrate accumulation and the suppression of photosynthesis. One of the possible mechanisms for the suppression of photosynthesis is a feedback inhibition of carbohydrate synthesis by a high carbohydrate level. For example, since sucrose synthesis is inhibited when sucrose accumulates in the leaf (Stitt et al. 1988, Stitt and Quick 1989, Foyer 1990), this feedback inhibition is thought to possibly induce a P_i-regeneration-limitation on photosynthesis because P_i is not recycled. Actually, however, this feedback inhibition leads to a stimulation of starch synthesis by shifts of P_i and metabolites, and the starch synthesis occurs without suppression of photosynthesis (Neuhaus et al. 1989, Stitt 1991). Thus, it is unlikely that a P_i-regeneration limitation comes into play. In addition, although another carbohydrate feedback signal may be generated in response to a limitation of phloem loading, transport in the phloem, or unloading of the phloem at the developing tissues, there is no evidence for this limitation on photosynthesis.

Gas-exchange studies also indirectly suggest that the suppression of photosynthesis by CO_2 enrichment is not caused by P_i-regeneration limitation. Since P_i-regeneration-limited photosynthesis is insensitive to elevating *pCi* (see Fig. 1), analysis of the *pCi* response of photosynthesis can deduce the involvement of this limitation. Sage (1994) reviewed more than 40 gas-exchange studies of the *pCi* response of photosynthesis. Although he found that the shape of the *pCi*-response curve of photosynthesis changes depending on the growth CO_2 level, most of the data suggest a decreased carboxylation capacity rather than a reduction in P_i-regeneration capacity.

Stitt (1991) proposed the existence of another feedback mechanism(s) by which the accumulation of carbohydrates directly or indirectly leads to a decrease in the amounts of key components of the photosynthetic apparatus. In fact, many studies of long-term CO_2 enrichment have shown a decrease in Rubisco content (Sage et al. 1989, Rowland-Bamford et al. 1991, Xu et al. 1994, Jacob et al. 1995, Rogers et al. 1996, Ghannoum et al. 1997, Vu et al. 1997, Nakano et al. 1997, Osborne et al. 1998, Sims et al. 1999). In addition, it has been obvious that the transcript levels of several photosynthetic genes including *rbc*S decrease in the plants grown at elevated CO_2 levels (Nie et al. 1995, van Oosten and Besford 1995, Gesch et al. 1998, Moore et al. 1998, Cheng et al. 1998). However, increased carbohydrates do not directly inhibit the expression of such genes and it seems to be associated with the metabolism of hexoses derived from sucrose hydrolysis by acid invertase (Goldschmidt and Huber 1992, Krapp et al. 1993, Moore et al. 1998). Although the phosphorylation of hexoses by hexokinase may involve the repression of photosynthetic gene expression (Sheen 1994, Jang et al. 1997,

Smeekens and Rook 1997), there are still many unknown aspects between the metabolism of hexoses and the repression of gene expression at elevated CO_2 (for a review, see Moore et al. 1999).

An apparent correlation between starch accumulation and suppression of photosynthesis has also been frequently observed (Nafziger and Koller 1976, Mauney et al. 1979, Azcon-Bieto 1983, Sàsek et al. 1985, Krapp and Stitt 1995). In many species, the increase of starch content by CO_2 enrichment seems to be relatively greater than that of soluble sugars (Morin et al. 1992, Den Hertog et al. 1996, Nakano et al. 1997, Poorter et al. 1997, Tissue et al. 1997, Moore et al. 1998). There have been some reports describing that extreme enlargement of starch grains may lead to physical damage of the chloroplasts (Cave et al. 1981, DeLucia et al. 1985, Yelle et al. 1989, Pritchard et al. 1997), but it is also possible that starch accumulation hinders CO_2 diffusion in the chloroplast (Nafziger and Koller 1976, Makino et al. 1994b, Nakano et al. 1999). A morphological modification of chloroplasts reserving excess starch may be an important factor in CO_2 transfer conductance because the conductance strongly depends on the chloroplast surface area adjacent to the plasmamembrane (von Caemmerer and Evans 1991). Nakano et al. (1999) have reported that the suppression of photosynthesis by excess starch is predominantly observed at *pCa* where CO_2 diffusion is limiting but it decreases or disappears under conditions of saturating CO_2. These results strongly suggest that excess starch leads to an increased diffusive resistance to CO_2. In addition, the reversibility of photosynthetic suppression with changes in starch content has been also observed in some studies with soybean (Thorne and Koller 1974), cucumber (Mayoral et al. 1985), cotton (Sasek et al. 1985) and bean (Nakano et al. 1999). However, all of these analyses are based on correlations and do not prove that any causal relationship exists. If the chloroplast movement regulates CO_2 diffusion between intercellular air spaces and the choroplast stroma (see above), starch accumulation in chloroplasts might be closely related to the chloroplast movement.

The extent to which starch and soluble sugars accumulation responds to CO_2 enrichment greatly depends on species. For example, bean (Sage et al. 1989, Nakano et al. 1998), cotton (Mauney et al. 1979, DeLucia et al. 1985), soybean (Nafziger and Koller 1976, Xu et al. 1994) and clover (Morin et al. 1992) preferentially accumulate starch, whereas in wheat (McKee and Woodward 1994), sunflower (Mauney et al. 1979) and rice (Nakano et al. 1997, 1998) the absolute amount of soluble sugars accumulated is greater than that of starch. For some of these plants, the decrease in photosynthesis by CO_2 enrichment can be found to be more strongly correlated with starch accumulation. Difference in the down-regulation of photosynthesis among species might be closely related to a difference in the absolute amount of starch accumulating in leaves (Nakano et al. 1997, 1998).

Decreases in Rubisco and leaf N contents—A decrease in Rubisco content or its activation state is commonly found in many C_3 plants grown under CO_2 enrichment (for a review, see Drake et al. 1997). However, this cannot be a determinant for the down-regulation of photosynthesis by CO_2 enrichment, because CO_2-enriched photosynthesis is not limited by Rubisco capacity but by electron-transport or P_i-regeneration capacity. Therefore, if the down-regulation of photosynthesis occurs during long-term CO_2 enrichment, either electron-transport components or key enzymes of starch and sucrose synthesis should decrease. However, many studies have shown that a decrease in Rubisco content by CO_2 enrichment is greater than that of other components such as electron transport components (Nie et al. 1995, Nakano et al. 1997, Theobald et al. 1998), key enzymes of sucrose synthesis (Nakano et al. 1997), soluble protein (Wong 1979, Ghannoum et al. 1997) or total leaf N (Sage et al. 1989, Rowland-Bamford et al. 1991, Rogers et al. 1996, Nakano et al. 1997, Osborne et al. 1998, Sims et al. 1998a). Although these findings suggest that a selective decrease in Rubisco occurs in the elevated-CO_2-grown plants, this is not necessarily right. Since the decrease in Rubisco content by CO_2 enrichment is always associated with a decrease in leaf N content, such a phenomenon makes it difficult to evaluate the change in N al-

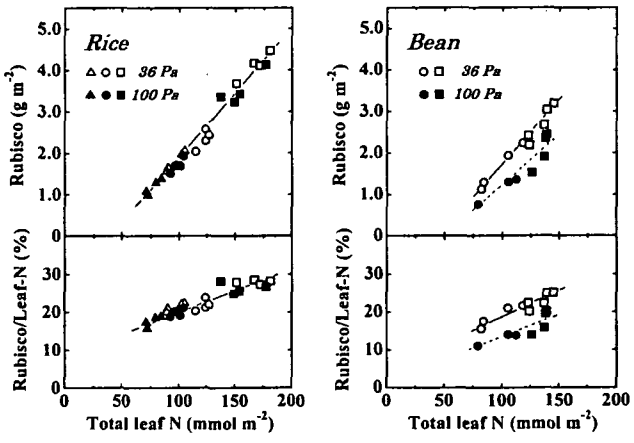

Fig. 2 Rubisco content (top) and its ratio to total leaf N content (bottom) versus total leaf N content in the uppermost, fully expanded leaves of rice (left panel) and bean (right panel). Rice plants were grown hydroponically at an irradiance of 850 μmol quanta $m^{-2} s^{-1}$ and a day/night temperature of 25/20°C under two *pCa* of 36 (open symbols) and 100 (closed symbols) and N concentrations of 0.5 (triangle), 2.0 (circle) and 8.0 (square) mM. Data are taken from figure 4 in Nakano et al. (1997). Bean plants were also grown hydroponically at an irradiance of 850 μmol quanta $m^{-2} s^{-1}$ and a day/night temperature of 23/18°C under two *pCa* of 36 (open symbols) and 100 (closed symbols) and N concentrations of 4.0 (circle) and 8.0 (square) mM. Data are taken from figure 2 in Nakano et al. (1998).

location to Rubisco and other components of photosynthesis by CO_2 enrichment. When N supply is low, Rubisco content is reduced relative to that of other components of photosynthesis, irrespective of growth CO_2 levels (Evans and Terashima 1988, Makino et al. 1992, 1994a). Nakano et al. (1997) grew rice plants with different N levels under two *pCa* of 36 and 100 Pa, and analyzed the relationship between Rubisco and leaf N contents (left panel in Fig. 2). Their results indicated no differences between the two CO_2 treatments in the relationship between Rubisco and leaf N contents. At the same time, they found that the decrease in photosynthesis by CO_2 enrichment can be simply accounted for by a decrease in the absolute amount of leaf N. In addition, although a relative decrease in Rubisco to other components of photosynthesis was found in the elevated-CO_2-grown plants, this was also the result of a decrease in total leaf N content by CO_2 enrichment. Similar results have been reported with wheat (Makino 1994, Theobald et al. 1998) and pea (Makino 1994). Thus, although long-term CO_2 enrichment leads to decreases in Rubisco content and photosynthesis, these are not the direct result of CO_2 enrichment but rather the result of a decrease in leaf N content induced by CO_2 enrichment.

However, these phenomena cannot be necessarily generalized. Nakano et al. (1998) did the same experiments on bean plants, and found that the ratio of Rubisco to leaf N content clearly decreased in the elevated-CO_2-grown plants (right panel in Fig. 2). A similar trend has also been found for lamb's-quarters and cabbage (Sage et al. 1989). Although these results suggest a selective decrease of Rubisco in these species, the real response of Rubisco to elevated CO_2 levels is still unknown. Sage et al. (1989) observed that the deactivated Rubisco in bean plants immediately after exposure to elevated CO_2 levels does not recover during the subsequent prolonged exposure to CO_2 enrichment. This means that CO_2 enrichment causes a reduction in both Rubisco content and RuBP regeneration capacity and that Rubisco remains excessive.

In summary, the long-term responses to CO_2 enrichment that are observed at the level of a single leaf can be attributed to either excess carbohydrate accumulation or decreased N content rather than direct responses to changing CO_2. Accumulation of carbohydrates may lead to the repression of photosynthetic gene expression and excess starch seems to hinder CO_2 diffusion. A decrease in photosynthesis by CO_2 enrichment is the result of a decrease in leaf N content. However, it is difficult to generalize such responses because photosynthesis observed at the level of a single leaf is closely related to the different growth strategies at the whole plant level. In the next section, we will discuss the variation in the response of photosynthesis to elevated CO_2 levels in relation to the growth at the whole plant level.

Photosynthesis and plant growth at elevated levels of CO_2

The down-regulation of photosynthesis by CO_2 enrichment is observed when the photosynthate exceeds the carbohydrate utilization for growth. However, there are some species showing little or no down-regulation of photosynthesis even when they are grown during long-term CO_2 enrichment and have excess carbohydrates. For example, potato (Sage et al. 1989) and radish (Usuda and Shimogawara 1998) do not show any down-regulation of photosynthesis whereas they genetically belong to starch-accumulating species. These species have the tuber or roots which can act as a large sink for carbohydrates. In radish, the biomass of the storage roots is markedly enhanced under CO_2 enrichment, and consequently no over-accumulation of carbohydrates is observed in leaves (Usuda and Shimogawara 1998). Similarly, in rice, the leaf sheaths can act as a temporary sink for carbohydrates, and the absolute amounts of carbohydrates in the leaf blades are considerably small (Nakano et al. 1995, 1997, 1998, Makino et al. 1997b, Watanabe et al. 1997). Therefore, the down-regulation of photosynthesis in rice may be small compared with that in bean (Sage et al. 1989), cotton (Mauney et al. 1979, DeLucia et al. 1985, Sasek et al. 1985) or soybean (Nafziger and Koller 1976, Xu et al. 1994). These species accumulate a great deal of carbohydrates in the leaves, especially as the starch in chloroplasts.

Another important point at the level of the whole plant is a decrease in leaf N content by CO_2 enrichment. This is commonly found in many species (Conroy and Hocking 1993, Delgado et al. 1994, Koike et al. 1995, Rogers et al. 1996, Roumet et al. 1996, Tissue et al. 1993, 1997, Poorter et al. 1997, Osborne et al. 1998, Sims et al. 1998a). This decrease in leaf N content is not due to dilution of N caused by relative increases in leaf area or plant mass. Makino et al. (1997a, 1999) reported that the decrease in leaf N content is the result of a change in N allocation at the morphogenic level of the whole plant. They grew rice plants with different N levels under two *pCa* of 36 and 100 Pa, and examined the effect of growth CO_2 levels on N allocation at the whole-plant level (Fig. 3). The N investment in the leaf blades of the plants grown in 100 Pa CO_2 decreases for all N treatments, whereas CO_2 enrichment enhances the allocation of N to the leaf sheaths and roots. This means that during long-term growth under conditions of elevated CO_2, plants reallocate N away from leaf blades to leaf sheaths and roots. In addition, since N invested into leaf blades is the most important source for photosynthesis in the whole plant, these results also indicate that plants regulate photosynthesis by changing N allocation at the whole plant level. Recently, Sims et al. (1998b) constructed a treatment system in which single soybean leaflets were exposed to CO_2 concentrations different from those experienced by the rest of the plant.

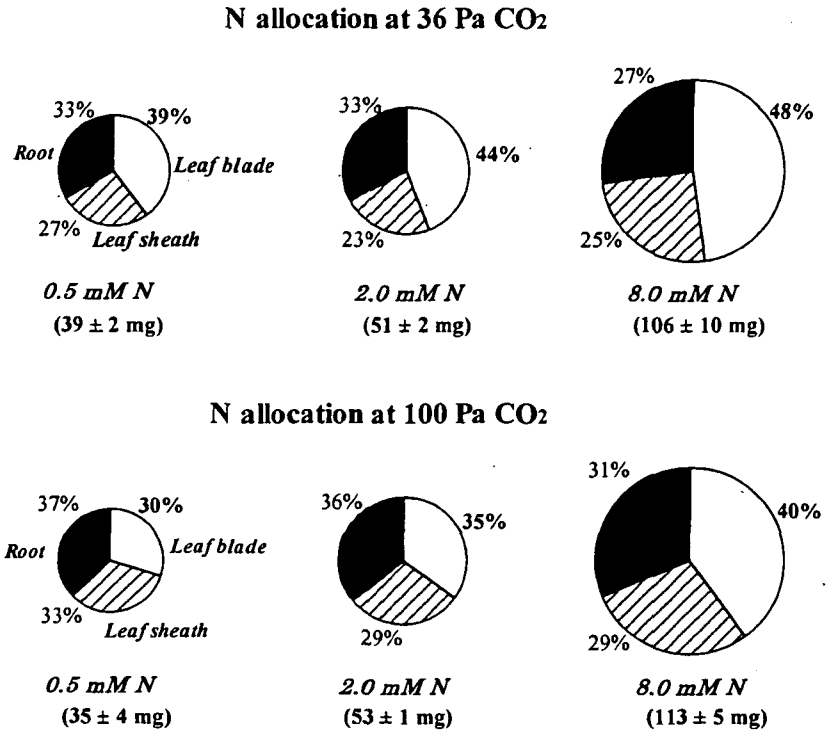

Fig. 3 N allocation of rice plants grown hydroponically under two *pCa* of 36 and 100 Pa at N concentrations of 0.5, 2.0 and 8.0 mM at the 70th d after germination. The N allocations to the leaf blade (open area), the leaf sheath (half-shaded area), and the root (closed area) are given as percentages of the total plant-N content on a plant basis which is shown by relative areas of pies. Values in parentheses are the total plant-N content on a plant basis (±SE, $n=8$). The stem did not develop at this stage, and its N content was negligible. Data are taken from figure 3 in Makino et al. (1997a).

According to their results, although the single leaflet treatments have dramatic effects on the carbohydrate contents of the treated leaflets, the Rubisco content is unaffected by such leaflet treatments and instead is related to the whole plant CO_2 environment. They did not measure leaf N content, but since the Rubisco content in a leaf is determined by N allocation to the leaf (Makino et al. 1984, 1997a, Nakano et al. 1997), their results also suggest the possibility that plants regulate leaf N content by changing N allocation at the whole plant level, irrespective of the carbohydrate content of the treated single leaflets.

Differences in the long-term response to CO_2 enrichment may be also related to differences in the sink-source status of the whole plant depending on the developmental stages. CO_2 enrichment frequently leads to a greater stimulation of biomass production in young seedlings than in matured plants such as soybean, cotton (Mauney et al. 1978), alfalfa (Baysdorfer and Bassham 1985), tobacco (Geiger et al. 1998) and rice (Makino et al. 1997a, Jitla et al. 1997). In rice, CO_2 enrichment stimulates N uptake during the seedling stage, but tends to suppress it during the matured stage (Makino et al. 1997a). Similarly, CO_2 enrichment promotes the development of the tillers, especially during early stage of growth, and decreases in leaf N content and photosynthesis are not observed (Makino et al. unpublished). In tobacco, carbohydrate levels are lower in young seedlings than in older plants (Geiger et al. 1998). Perhaps, growth during the seedling stage is 'source-limited' and the carbohydrates increased by CO_2 enrichment can be efficiently utilized for additional sink such as the development of new tillers or secondary shoots.

Elevated CO_2 levels accelerate development of the whole plant such as radish (Usuda and Shimogawara 1998) and tobacco (Masle et al. 1993). On the other hand, the developmental rate of wheat (Mitchell et al. 1993) and rice (Makino et al. 1997a, 1999) is not affected by CO_2 enrichment (although the heading time was 2 to 4 d earlier in the elevated-CO_2-grown rice, Makino et al. unpublished). Miller et al. (1997) reported that the down-regulation of photosynthesis by CO_2 enrichment in tobacco appears to be the result of a shift in the timing of the natural ontogenetic decline of photosynthesis to an earlier onset associated with leaf senescence. Although this seems to be related to a developmental acceleration at the whole plant level in tobacco, an acceleration of leaf senescence has been frequently observed in other species such as wheat (Nie et al. 1995, Osborne et al. 1998, Theobald et al. 1998), sunflower (Sims et al. 1999), *Nardus strica* (Cook et al.

1998), and rice (Makino et al. 1999). This means that an acceleration of leaf senescence by CO_2 enrichment is independent of the developmental acceleration at the whole-plant level. In rice, although leaf senescence is accelerated by CO_2 enrichment, there is no shift in the timing of the maximum photosynthetic stage of leaf ontogeny (Makino et al. 1999). Similar results can be also found in wheat (Theobald et al. 1998). Thus, different responses of photosynthesis to CO_2 observed at the level of a single leaf may result from different growth strategies of the whole plant under conditions of CO_2 enrichment.

Concluding remarks

We have described the effect of elevated levels of CO_2 on the photosynthetic mechanisms in relation to the whole-plant growth in higher C_3 plants. The responses to CO_2 in plants are very variable, but we cannot find any positive acclimation for plant growth under conditions of CO_2 enrichment. Since changes in the atmospheric CO_2 levels during these 100,000 years before the Industrial Revolution have been very small over the generation of individual plants, it might be natural that plants lack the ability to acclimate to elevated levels of CO_2. In addition, since CO_2 enrichment is not a stress for plants, plants might have lacked the need to acclimate to elevating CO_2. The responses described here are attributed to secondary responses related to either excess carbohydrate accumulation or decreased N content rather than direct responses to CO_2. Although it is obvious that the carbohydrate metabolism is closely related to the development of the photosynthetic apparatus, no CO_2 sensor has been found in plants. Various responses of photosynthesis to CO_2 result from manifold growth strategies of plants under conditions of CO_2 enrichment. We cannot elucidate the real response of photosynthesis to an environmental change without relating to the growth at the whole plant level.

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References

Assmann, S.M. (1999) *Plant Cell Environ.* 22: 629–637.
Azcon-Bieto, J. (1983) *Plant Physiol.* 73: 681–686.
Baysdorfer, C. and Bassham, J.A. (1985) *Plant Physiol.* 77: 313–317.
Campbell, W.J., Allen, L.H., Jr. and Bowes, G. (1988) *Plant Physiol.* 88: 1310–1316.
Cave, G., Tolley, L.C. and Strain, B.R. (1981) *Physiol. Plant.* 51: 171–174.
Cheng, S.-H., Moore, d.B. and Seemann, J.R. (1998) *Plant Physiol.* 116: 715–723.
Conroy, J. and Hocking, P. (1993) *Physiol. Plant.* 89: 570–576.
Cook, A.C., Tissue, D.T., Roberts, S.W. and Oechel, W.C. (1998) *Plant Cell Environ.* 21: 417–425.
Delgado, E., Mitchell, R.A.C., Parry, M.A.J., Driscoll, S.P., Mitchell, V.J. and Lawlor, D.W. (1994) *Plant Cell Environ.* 17: 1205–1213.
DeLucia, E.H., Sasek, T.W. and Strain, B.R. (1985) *Photosynth. Res.* 7: 175–184.
Den Hertog, J., Stulen, I., Fonseca, F. and Delea, P. (1996) *Physiol. Plant.* 98: 77–88.
Drake, B.G., Gonzalez-Meler, M.A. and Long, S.P. (1997) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48: 609–639.
Evans, J.R. (1986) *Planta* 167: 351–358.
Evans, J.R. and Terashima, I. (1988) *Plant Cell Physiol.* 29: 157–165.
Evans, J.R. and von Caemmerer, S. (1996) *Plant Physiol.* 110: 339–346.
Farquhar, G.D. and von Caemmerer, S. (1982) *In* Encyclopedia of Plant Physiology. Vol. 12B. Water Relations and Carbon Assimilation. Edited by Nobel, P.S., Osmond, C.B. and Ziegler, H. pp. 549–587. Springer-Verlag, Berlin.
Farquhar, G.D., von Caemmerer, S. and Berry, J.A. (1980) *Planta* 149: 178–190.
Foyer, C.H. (1990) *Plant Physiol. Biochem.* 28: 151–160.
Geiger, M., Walch-Liu, P., Engels, C., Harnecker, J., Schulze, E.-D., Ludewig, F., Sonnewald, U., Scheible, W.-R. and Stitt, M. (1998) *Plant Cell Environ.* 21: 253–268.
Gesch, R.W., Boote, K.J., Vu, J.C.V., Allen, L.H., Jr. and Bowes, G. (1998) *Plant Physiol.* 118: 521–529.
Ghannoun, O., von Caemmerer, S., Balow, E.W.R. and Conroy, J.P. (1997) *Aust. J. Plant Physiol.* 24: 227–237.
Goldschmidt, E.E. and Huber, S.C. (1992) *Plant Physiol.* 99: 1443–1448.
Haupt, W. and Scheuerlein, R. (1990) *Plant Cell Environ.* 13: 595–614.
Jacob, J., Greitner, C. and Drake, B.G. (1995) *Plant Cell Environ.* 18: 875–884.
Jang, J.-C., Leon, P., Zhou, L. and Sheen, J. (1997) *Plant Cell* 9: 5–19.
Jarvis, A.J., Mansfield, T.A. and Davies, W.J. (1999) *Plant Cell Environ.* 22: 639–648.
Jitla, D.S., Rogers, G.S., Seneweera, S.P., Basra, A.S., Oldfield, R.J. and Conroy, J.P. (1997) *Plant Physiol.* 115: 15–22.
Kadota, A. and Wada, M. (1992) *Protoplasma* 167: 97–107.
Koike, T., Kohda, H., Mori, S., Takahashi, K., Inoue, M.T. and Lei, T. (1995) *Plant Species Biol.* 10: 95–101.
Krapp, A., Hofmann, B., Schafer, C. and Stitt, M. (1993) *Plant J.* 3: 817–828.
Krapp, A. and Stitt, M. (1995) *Planta* 195: 313–323.
Lorimer, G.H. (1981) *Annu. Rev. Plant Physiol.* 32: 349–383.
Makino, A. (1994) *J. Plant Res.* 107: 79–84.
Makino, A., Harada, M., Kaneko, K., Mae, T., Shimada, T. and Yamamoto, N. (1999) *Aust. J. Plant Physiol.* in press.
Makino, A., Harada, M., Sato, T. and Mae, T. (1997a) *Plant Physiol.* 115: 199–203.
Makino, A., Mae, T. and Ohira, K. (1984) *Plant Cell Physiol.* 25: 429–437.
Makino, A., Mae, T. and Ohira, K. (1985) *Planta* 166: 414–420.
Makino, A., Nakano, H. and Mae, T. (1994a) *Plant Physiol.* 105: 173–179.
Makino, A., Nakano, H. and Mae, T. (1994b) *Plant Physiol.* 105: 1213–1238.
Makino, A., Sakashita, H., Hidema, J., Mae, T., Ojima, K. and Osmond, B. (1992) *Plant Physiol.* 100: 1737–1743.
Makino, A., Sato, T., Nakano, H. and Mae, T. (1997b) *Planta* 203: 390–398.
Makino, A., Shimada, T., Tokumi, S., Kaneko, K., Matsuoka, M., Shimamoto, K., Nakano, H., Miyao-Tokutomi, M., Mae, T. and Yamamoto, N. (1997c) *Plant Physiol.* 114: 483–491.
Masle, J., Hudson, G.S. and Badger, M.R. (1993) *Plant Physiol.* 103: 1075–1088.
Mauney, J.R., Fry, K.E. and Guinn, G. (1978) *Crop Sci.* 18: 259–263.
Mauney, J.R., Guinn, G., Fry, K.E. and Hesketh, J.D. (1979) *Photosynthetica* 13: 260–266.
Mayoral, M.L., Plaut, Z. and Reinhold, L. (1985) *Plant Physiol.* 77: 712–717.
McKee, I.F. and Woodward, F.I. (1994) *Plant Cell Environ.* 17: 853–859.
Miller, A., Tsai, C.-H., Hemphill, D., Endres, M., Rodermel, S. and Spalding, M. (1997) *Plant Physiol.* 115: 1195–1200.
Mitchell, R.A.C., Mitchell, V.J., Driscoll, S.P., Franklin, J. and Lawlor,

D.W. (1993) *Plant Cell Environ.* 16: 521–529.
Moore, B.d., Cheng, S.-H., Rice, J. and Seemann, J.R. (1998) *Plant Cell Environ.* 21: 905–915.
Moore, B.d., Cheng, S.-H., Sims, D. and Seemann, J.R. (1999) *Plant Cell Environ.* 22: 567–582.
Morin, F., Andre, M. and Bestsche, T. (1992) *Plant Physiol.* 99: 89–95.
Nafziger, E.D. and Koller, R.M. (1976) *Plant Physiol.* 57: 560–563.
Nakano, H., Makino, A. and Mae, T. (1995) *Plant Cell Physiol.* 36: 653–659.
Nakano, H., Makino, A. and Mae, T. (1997) *Plant Physiol.* 115: 191–198.
Nakano, H., Makino, A. and Mae, T. (1998) *In* Photosynthesis: Mechanisms and Effects. Vol. 5. Edited by Garab, G. pp. 3391–3394. Kluwer Academic Publishers, Dorderecht.
Nakano, H., Muramatsu, S., Makino, A. and Mae, T. (1999) *Aust. J. Plant Physiol.* in press.
Neuhaus, H.E., Kruckeberg, A.L., Feil, R. and Stitt, M. (1989) *Planta* 178: 110–122.
Nie, G.Y., Long, S.P., Garcia, R.L., Kimball, B.A., LaMorte, R.L., Pinter, P.J., Jr., Wall, G.W. and Webber, A.N. (1995) *Plant Cell Environ.* 18: 855–864.
Osborne, C.P., LaRoche, J., Garcia, R.L., Kimball, B.A., Wall, G.W., Pinter, P.J., Jr., LaMorte, R.L., Hendrey, G.R. and Long, S.P. (1998) *Plant Physiol.* 117: 1037–1045.
Poorter, H., Van Berkel, Y., Baxter, R., den Hertog, J., Dijkstra, P., Gifford, R.M., Griffin, K.L., Roumet, C., Roy, J. and Wong, S.C. (1997) *Plant Cell Environ.* 20: 472–482.
Pritchard, S.G., Peterson, C.M., Prior, S.A. and Rogers, H.H. (1997) *Plant Cell Environ.* 20: 461–471.
Rogers, G.S., Milham, P.J., Gillings, M. and Conroy, J. (1996) *Aust. J. Plant Physiol.* 23: 253–264.
Roumet, C., Bel, M.P., Sonie, L., Jardon, F. and Roy, J. (1996) *New Phytol.* 133: 595–603.
Rowland-Bamford, A.J., Baker, J.T., Allen, L.H., Jr. and Bowes, G. (1991) *Plant Cell Environ.* 14: 577–583.
Sage, R.F. (1990) *Plant Physiol.* 94: 1728–1734.
Sage, R.F. (1994) *Photosynth. Res.* 39: 351–368.
Sage, R.F. and Sharkey, T.D. (1987) *Plant Physiol.* 84: 658–664.
Sage, R.F., Sharkey, T.D. and Seemann, J.R. (1988) *Planta* 174: 407–416.
Sage, R.F., Sharkey, T.D. and Seemann, J.R. (1989) *Plant Physiol.* 89: 590–596.
Sage, R.F., Sharkey, T.D. and Seemann, J.R. (1990) *Plant Physiol.* 94: 1735–1742.
Sasek, T.W., DeLucia, E.H. and Strain, B.R. (1985) *Plant Physiol.* 78: 619–622.
Sharkey, T.D. (1985a) *Bot. Rev.* 51: 53–105.
Sharkey, T.D. (1985b) *Plant Physiol.* 78: 71–75.
Sheen, J. (1994) *Photosynth. Res.* 39: 427–438.
Sicher, R.C. and Kremer, D.F. (1994) *Plant Physiol.* 92: 383–388.
Sicher, R.C., Kremer, D.F. and Bunce, J.A. (1995) *Photosynth. Res.* 46: 409–417.
Sims, D.A., Cheng, W., Luo, Y. and Seemann, J.R. (1999) *J. Exp. Bot.* 50: 645–653.
Sims, D.A., Luo, Y. and Seemann, J.R. (1998a) *Plant Cell Environ.* 21: 945–952.
Sims, D.A., Luo, Y. and Seemann, J.R. (1998b) *Plant Cell Environ.* 21: 1189–1196.
Sivak, M.N. and Walker, D.A. (1986) *Plant Physiol. Biochem.* 25: 635–648.
Smeekens, S. and Rook, F. (1997) *Plant Physiol.* 115: 7–13.
Socias, F.X., Medrano, H. and Sharkey, T.D. (1993) *Plant Cell Environ.* 16: 81–86.
Stitt, M. (1986) *Plant Physiol.* 81: 1115–1222.
Stitt, M. (1991) *Plant Cell Environ.* 14: 741–762.
Stitt, M. and Krapp, A. (1999) *Plant Cell Environ.* 22: 583–621.
Stitt, M. and Quick, W.P. (1989) *Physiol. Plant.* 77: 633–641.
Stitt, M., Wilke, I., Feil, R. and Heldt, H.W. (1988) *Planta* 174: 217–230.
Terashima, I., Ishibashi, M., Ono, K. and Hikosaka, K. (1995) *In* Photosynthesis from Light to Biosphere. Vol. 5. Edited by Mathis, P. pp. 537–542. Kluwer Academic Publishers, Dordrecht.
Theobald, J.C., Mitchell, R.A.C., Parry, M.A.J. and Lawlor, D.W. (1998) *Plant Physiol.* 118: 945–955.
Thorne, J.M. and Koller, R.M. (1974) *Plant Physiol.* 54: 201–207.
Tissue, D.T., Thomas, R.B. and Strain, B.R. (1993) *Plant Cell Environ.* 16: 859–865.
Tissue, D.T., Thomas, R.B. and Strain, B.R. (1997) *Plant Cell Environ.* 20: 1123–1134.
Usuda, H. and Shimogawara, K. (1998) *Plant Cell Physiol.* 39: 1–7.
Van Oosten, J.-J. and Besford, R.T. (1995) *Plant Cell Environ.* 18: 1253–1266.
Von Caemmerer, S. and Edmondson, D.L. (1986) *Aust. J. Plant Physiol.* 13: 669–688.
Von Caemmerer, S. and Evans, J.R. (1991) *Aust. J. Plant Physiol.* 18: 287–305.
Von Caemmerer, S. and Farquhar, G.D. (1981) *Planta* 153: 376–387.
Vu, J.C.V., Allen, L.H., Jr., Boote, K.J. and Bowes, G. (1997) *Plant Cell Environ.* 20: 68–76.
Xu, D.-Q., Gifford, R.M. and Chow, W.S. (1994) *Plant Physiol.* 106: 661–671.
Watanabe, Y., Nakamura, Y. and Ishii, R. (1997) *Aust. J. Plant Physiol.* 24: 563–569.
Wong, S.C. (1979) *Oecologia* 44: 68–74.
Yelle, S., Beeson, R.C., Jr., Trudel, M.J. and Gosselin, A. (1989) *Plant Physiol.* 90: 1465–1472.

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