Increasing crop productivity to meet burgeoning human food demand is challenging under changing environmental conditions. Since industrial revolution atmospheric CO2 levels have linearly increased. Developing crop varieties with increased utilization of CO2 for photosynthesis is an urgent requirement to cope with the irreversible rise of atmospheric CO2 and achieve higher food production. The primary effects of elevated CO2 levels in most crop plants, particularly C3 plants, include increased biomass accumulation, although initial stimulation of net photosynthesis rate is only temporal and plants fail to sustain the maximal stimulation, a phenomenon known as photosynthesis acclimation. Despite this acclimation, grain yield is known to marginally increase under elevated CO2. The yield potential of C3 crops is limited by their capacity to exploit sufficient carbon. The “C fertilization” through elevated CO2 levels could potentially be used for substantial yield increase. Rubisco is the rate-limiting enzyme in photosynthesis and its activity is largely affected by atmospheric CO2 and nitrogen availability. In addition, maintenance of the C/N ratio is pivotal for various growth and development processes in plants governing yield and seed quality. For maximizing the benefits of elevated CO2 raising plant nitrogen pools will be necessary as part of maintaining an optimal C/N balance. In this review, we discuss potential causes for the stagnation in yield increases under elevated CO2 levels and explore possibilities to overcome this limitation by improved photosynthetic capacity and enhanced nitrogen use efficiency. Opportunities of engineering nitrogen uptake, assimilatory, and responsive genes are also discussed that could ensure optimal nitrogen allocation towards expanding source and sink tissues. This might aver photosynthetic acclimation partially or completely and drive for improved crop production under elevated CO2 levels.

Keywords: photosynthesis, nitrogen use efficiency, Rubisco, carbon, nitrogen, elevated CO2, yield
2009). Simultaneous improvement of both C and N utilization efficiencies is of utmost importance, given the rising atmospheric CO2 levels and the necessity to lower input costs and reduce environmental pollution due to excessive use of nitrogenous fertilizers. Therefore, fine-tuning of genetic changes leading to metabolic adjustments of both C and N will be required in order to effectively harness excess C from elevated atmospheric CO2 and to simultaneously maintain an optimal C/N balance. The primary effects of elevated CO2 levels in most crop plants (especially C3 plants) include increased plant biomass accumulation, although initial stimulation of net photosynthesis rates for most C3 plants is only temporal, and they fail to sustain the maximum stimulation (though higher than ambient CO2 level) over longer exposure periods (months to years). This phenomenon is called CO2 or photosynthesis acclimation (Long et al., 2004; Reich et al., 2006; Böhm et al., 2010). This photosynthesis acclimation (initial stimulation followed by a partial reversal or stabilization at a lower rate) under elevated CO2 is accompanied by a decrease in carboxylation of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), a decrease in N concentration, a reduced stomatal conductance, and an increase of starch accumulation (Figure 1; Nakano et al., 1997; Geiger et al., 1999; Stitt and Kräpp, 1999; Böhm et al., 2002, 2010; Long et al., 2004; Ainsworth and Long, 2005; Leskey et al., 2009).

In C3 plants, Rubisco is the key chloroplast enzyme (comprising ~50% of total cellular proteins) involved in photosynthesis through catalyzing the carboxylation of ribulose-1,5-bisphosphate (RuBP) during capture and fixation of atmospheric CO2. This CO2 is later converted into sugars, the major building blocks for plants. In addition to the involvement of Rubisco in C metabolism, it is also a major storage protein for N (Makino and Osmond, 1991; Mae et al., 1993). This stored N is further utilized by the plants’ reproductive components when Rubisco degradation is initiated during leaf senescence. The consequences of elevated CO2 on plants are 2-fold; the decreased Rubisco level becomes a rate-limiting factor for photosynthetic efficiency compounded by a reduction in the available N pool. Genetically engineered plants producing increased levels of Rubisco protein could potentially improve CO2 fixation. However, plants under these conditions would require additional N for increased Rubisco production. Additionally, maintenance of an optimum C/N ratio within the plant is essential for efficient metabolism of C and N, optimal growth, and sustained quantitative and qualitative yield. High C status (specifically carbohydrates) due to increased CO2 levels would increase the C/N ratio with lower N levels resulting in lower protein content, thus reducing grain quality particularly in cereal crops.

There is mounting evidence that the yield potential of many crops is limited by their capacity to exploit sufficient C during their lifecycle, limiting grain size and quantity (Fischer et al., 1998). "C fertilization" through increased CO2 levels would be ideal for yield increase; however, photosynthetic acclimation restricts the plants’ ability to exploit elevated atmospheric CO2. In this review, we discuss the underlying causes of this stagnation in yield progress and explore the possibilities of improving the photosynthetic machinery in plants, combined with enhanced nitrogen use efficiency (NUE) under elevated CO2 conditions. Engineering N uptake, assimilatory and responsive genes would ensure optimal N allocation toward expanding source and sink tissues under elevated CO2 levels as well as improving grain yield and quality.

**C3 AND C4 PHOTOSYNTHETIC MECHANISMS**

Photosynthesis is the process whereby light is harvested by the chloroplast thylakoids of the leaf and other photosynthetic structures. The resultant chemical energy (ATP and NADPH) is used to fix atmospheric CO2, either directly via Rubisco (C3 photosynthesis), or indirectly after primary fixation by phosphoenolpyruvate carboxylase (PEPC). C fixed through this mechanism is subsequently re-released into adjacent cells which are not in direct communication with atmospheric CO2 (C4 photosynthesis). The majority of crop species (rice, wheat, grain legumes, canola, and all root crops) and ~85% of terrestrial plants use C3 photosynthesis, while C4 crops are a minority, represented predominantly by maize, sorghum, and sugarcane among economically important crops (Ehleringer et al., 1991).

The Rubisco enzyme, which is fundamental to C fixation in both C3 and C4 plants, displays a high affinity to O2, and its inability to distinguish it from the CO2 molecule results in unnecessary O2 uptake, especially under hot and arid conditions. This oxygenation activity produces phosphoglycolate molecules, which are then broken down in a process referred to as photorespiration, an energy-consuming and wasteful process (Kajala et al., 2011). Photorespiration has been identified as the bottleneck preventing C3 plants from achieving full photosynthetic potential due to competition between CO2 and O2 at the C fixation site on the Rubisco enzyme. Whereas, C4 photosynthesis evolved to ameliorate photorespiration by utilizing two distinct cell types not
in contrast, as C3 plants are not photosynthetically saturated at current CO2 ratios of CO2 to O2 at the site of fixation in the chloroplast, favoring PCR over PCO, and thus photosynthetic rates are increased in C4 plants compared to C3 plants. C4 plants can photosynthesize with ~50% greater water use efficiency, as C4 photosynthesis can assimilate an equivalent amount of CO2 with only half the stomatal conductance (Sage and Kubien, 2003; von Caemmerer and Furbank, 2003). Under N-limiting conditions, C4 plants also out-compete C3 plants, as they require less Rubisco to harness a similar amount of C due to increased photosynthetic efficiency (Sage and Kubien, 2003).

Since C4 plants are photosynthetically saturated at current CO2 conditions, predicted rises in atmospheric CO2 would have no major impact on their C fixation rate, biomass production, and yield (Figure 2; Cure and Acock, 1986; Ainsworth and Long, 2005). In contrast, as C3 plants are not photosynthetically saturated at present CO2 levels, photosynthesis, biomass, and subsequent yields should increase with elevated atmospheric CO2. The understanding of the biochemical and molecular nature of C3 and C4 photosynthesis provides a valuable tool for crop improvement in the twenty-first century, particularly with respect to improving C assimilation in C3 plants and reducing the impact of photosynthetic acclimation.

**IMPACTS OF ELEVATED CO2**

**LEAF PHOTOSYNTHESIS, GROWTH, AND YIELD**

The present atmospheric CO2 concentration of 390 μL CO2 L−1 limits the rate of photosynthesis in C3 plants (Farquhar et al., 1980; Farquhar and Sharkey, 1982), and presumably lower concentrations of CO2 in the recent past were even more limiting. Laboratory and field studies have shown that photosynthetic rates of C3 plants were approximately doubled when plants grown at about 380 μL CO2 L−1 were exposed to 700 μL CO2 L−1 (Ainsworth and Long, 2005). This increase in photosynthetic rate as atmospheric CO2 rises is primarily due to increase in Rubisco carboxylation capacity. Rubisco has an affinity for O2 as well as CO2 (Badger and Price, 2003); consequently, at 21% O2 and 390 μL CO2 L−1, a considerable amount of energy is wasted in the photorespiratory carbon oxidation cycle (PCO). This reduces photosynthetic rates by about 40% from the optimum level (Sharkey, 1985). Increasing the ambient CO2 concentration increases the ratio of CO2 to O2 at the site of fixation in the chloroplast, favoring PCR over PCO, and thus photosynthetic rates are increased in C3 plants. The limitation of photosynthesis imposed by Rubisco is referred to as the limitation due to supply and utilization of CO2 (Farquhar and Sharkey, 1982). Two other limitations were also identified: the supply and utilization of light and the utilization of triose phosphate. The former can be caused by low photon flux densities or inability to convert light energy to chemical energy. Triose phosphate is the end product of photosynthesis and can be formed into starches and sugars or utilized as a direct source of chemical energy. Limitation occurs when there are insufficient sinks for sucrose (Stitt and Schulze, 1994), thus reducing conversion to sugar and inhibiting photosynthesis. These three limitations to leaf photosynthesis were first identified in studies that were grown at a given CO2 concentration and then transferred to different CO2 concentration during measurement of photosynthesis (Drake et al., 1997). However, when plants are exposed to high CO2 for extended period, the photosynthetic rates slow down due to the so-called “acclimation” response (Long et al., 2004; Reich et al., 2006). This is thought to result from direct effects of sucrose on the transcription of genes encoding proteins involved in CO2 fixation and electron transport activity (Moore et al., 1999).

The effect of elevated CO2 on plant growth and yield has been studied in both controlled and field conditions, with the latter referred to as the Free Air Carbon dioxide Enrichment (FACE) system. The controlled conditions might produce larger artifacts, whereas FACE produces an environment similar to field conditions. The differential plant response under the two conditions has been reported. For example, Ainsworth et al. (2008) suggested
a 14% yield increase in FACE and a 31% increase in controlled conditions in different plant species when CO₂ was raised from ~373 to ~570 µmol mol⁻¹. Generally, elevated CO₂ increases photosynthesis, resulting in increased dry matter accumulation, leaf area, and plant height in trees and shrubs and to some extent in C₃ plants (Figure 2; Ainsworth and Long, 2005). The yield increase in C₄ crops under elevated CO₂ is variable and dependent on other environmental factors such as water, temperature, and soil N (Ainsworth and Long, 2005). Irrespective of photosynthetic machinery, a yield increase requires a concomitant increase in sink capacity to match the source activity. The initial response of C₃ plants to elevated CO₂ is an increase in photosynthetic rate; however, due to the acclimation phenomenon this stimulation is not always maintained at the maximal level when plants are exposed to elevated CO₂ for a longer period. This partial reversal of photosynthesis and settling at lower than maximal level could be ascribed to (i) reduced stomatal conductance resulting in depletion of intercellular CO₂ leading to reduced CO₂ supply to the photosynthetic machinery, and (ii) reduced rates of electron transport to Rubisco carboxylation (Figure 1 and also discussed in the following sub-section). Lower activation state and reduced concentration of Rubisco leads to changes in C assimilation and alters the whole plant N metabolism. Thereby, biochemical adjustments occur from the cellular to whole plant level in response to elevated CO₂, accompanied by growth, development, and yield changes.

MOLECULAR CHANGES IN PLANTS
Rubisco is the rate-limiting enzyme in photosynthesis and its synthesis and degradation is affected by environmental factors such as temperature, light intensity, soil N, and atmospheric CO₂. Prolonged exposure to elevated CO₂ results in reduced Rubisco content and Rubisco activity (Moore et al., 1999; Aranjuelo et al., 2011; Seneweera et al., 2011). A concomitant reduction in the transcript level of genes encoding proteins involved in photosynthesis, including small subunit of Rubisco (RbcS), large subunit of Rubisco (RbcL), and Rubisco activase (Rca), has been observed in different plants (Nie et al., 1995; Cheng et al., 1998; Moore et al., 1998, 1999; Stitt and Krapp, 1999). In contrast, in expanding rice leaf blades there was no significant difference in RbcS transcript level between ambient and elevated CO₂ levels (Aoki et al., 2004). This can be correlated with changes in Rubisco concentration during leaf development, with a rapid increase in Rubisco protein during leaf expansion, reaching a maximum when the leaf is fully expanded and a gradual decline with the onset of leaf senescence (Seneweera and Connoly, 2005; Imai et al., 2008). The decline in Rubisco and subsequent photosynthesis acclimation in plants under elevated CO₂ could be attributed to two processes. It could be due to carbohydrate sink limitation since plants grown under CO₂ enrichment initially assimilate more CO₂ than they can incorporate in their sinks tissues, and as a feedback response plants diminish CO₂ assimilation by reduced Rubisco and other proteins (Long et al., 2004). Previous reviews have reported that feedback repression of the RbcS and RbcL genes by soluble carbohydrates accumulation leads to a decline in Rubisco protein levels (Moore et al., 1999). Alternatively the C/N ratio usually increases under elevated CO₂ (Geiger et al., 1999; Loo et al., 2004; Taub and Wang, 2008; Bloom et al., 2010), since N is a key constituent of Rubisco and it becomes a rate-limiting factor for Rubisco synthesis (Nakano et al., 1997; Seneweera et al., 2011).

NUTRITIONAL CHANGES IN PLANTS
Elevated CO₂ stimulates higher photosynthesis and an increased growth rate, which is required to match with an increased demand for nutrients. This may vary between plant species, nutrient availability, and the nutrient element in question. Among different nutrient elements, maintaining the C/N balance is important for optimal plant growth. For instance, under a higher C/N ratio in soil or growth media, there is a reduced uptake of N in plants, leading to reduced grain quality in cereals due to lower grain protein content. In cereals such as wheat, rice, and barley, a decrease of up to 15% grain protein was observed under elevated CO₂ with an overall decrease in amino acid concentrations (Taub et al., 2008; Wieser et al., 2008; Hög et al., 2009). A decrease in cereal grain quality and a reduced protein composition may have serious health and economic implications. The spatial leaf N content has a strong correlation with Rubisco content in rice leaves, suggesting their inter-dependency with net photosynthetic rates (Seneweera, 2011). Leaf N allocation clearly declines under elevated CO₂ accompanied by lower chlorophyll content, as both are closely linked (Figure 1; Conroy and Hocking, 1993; Nakano et al., 1997; Ainsworth and Long, 2005; Leakey et al., 2009), also discussed in a later section. Other macro- and micro-nutrient concentrations change under elevated CO₂ conditions, though with lesser implications compared to N (Hög et al., 2009; Erbs et al., 2010). Potassium and phosphorous contents can increase or decrease depending upon growth conditions. Significantly lower levels of sodium, calcium, magnesium, sulfur, iron, zinc, manganese, and aluminum contents have been observed in wheat grain under elevated compared to ambient CO₂ (Hög and Fangmeier, 2008).

STRATEGIES FOR IMPROVING PHOTOSYNTHETIC RATES IN C₃ PLANTS
Increase in net photosynthesis per unit leaf area is important for increasing crop production to meet the world food demand. To improve photosynthesis rates in C₃ plants several approaches have been used, for example introducing C₄ like characteristics into C₃ cells (Kajula et al., 2011; Miyao et al., 2011; Petersansel, 2011); introducing a CO₂/HCO₃ pump protein into chloroplast membranes from cyanobacteria (Price et al., 2008); introducing new catalytic pathways into plastids that bypass the photorespiratory recycling Rubisco oxygenation product, 2-phosphoglycerate, and concomitantly releasing CO₂ into the stroma (Kebeish et al., 2007); and also improving the Rubisco kinetic characteristics. Some opportunities to improve photosynthetic efficiency in C₃ plants are discussed here.

C₄ photosynthesis has been identified to be evolving independently at least 66 times in 19 different families of angiosperms (Sage et al., 2011) with 21 of these lineages displaying the intermediate C₃–C₄ photosynthetic characteristics (Brown and Hattersley, 1988; Edwards et al., 2004). The evolution of C₄ from C₃ photosynthesis involves a number of intermediate steps, while the enzymes and structures present in C₄ plants are also present in
were found to have a C4 photosynthesis system contained within C3 cells. However, two species of the family Chenopodiaceae very similar to those in C4; however their expression level is very low are thought to serve housekeeping functions (Kajala et al., 2011). Overexpression of native C3 genes or homologous C4 genes could possibly be useful for improving photosynthetic efficiency in C3 plants. Attempts have been made to engineer single-celled and two-celled photosynthetic pathways with expression of specific genes into rice (Kajala et al., 2011; Miyao et al., 2011). Four C4 genes, PEPC, malate dehydrogenase (MDH), NADP-malic enzyme (NADP-ME), and NADP-malate dehydrogenase (NADP-MDH) have been engineered in rice (Tanguchi et al., 2008; Miyao et al., 2011). However, a functional C4 cycle in leaves of C3 species has not yet achieved. In addition, the negative effects of engineering C4 genes in C3 plants have been reported, such as overexpression of maize NADP-ME in rice that led to enhanced photoinhibition of photosynthesis and pleiotropic effects (Tsuda et al., 2001), stunted transgenic plants due to generation of futile cycles and improper circadian regulation of genes (Tanguchi et al., 2008; Kajala et al., 2011). To achieve C4 photosynthetic pathway operation in C3 plants is an enormous challenge and would require: careful selection and engineering of multiple genes encoding for both C4 photosynthetic genes and transporters of C4 metabolites, driving the optimal expression of genes, site-specific expression of selected genes such as in MC or BSC, choice of coding sequence since C4 genes have acquired changes in coding regions during evolutionary process, and proper regulation of C4 enzymes (Kajala et al., 2011; Miyao et al., 2011; Peterhansel, 2011).

Cyanobacteria are a phylum of bacteria that obtain energy through photosynthesis and are the ancestors of chloroplasts in eukaryotic cells (Raven and Allen, 2003). Cyanobacteria are highly efficient for producing biomass from inorganic C (Nogales et al., 1996; Riviere-Rolland et al., 1996; Rogers et al., 1996; Geiger et al., 1999). The decrease of N content in plant biomass at elevated CO2 compared to ambient CO2 is usually within the range of 10–15% (Figure 1; Jablonski et al., 2002; Kim et al., 2003; Martin et al., 2002; Malamy, 2005; Wingler et al., 2006; Gutierrez et al., 2007). Also, the exogenous supply of C as sucrose to N-limiting plants led to accumulation of higher carbohydrate and a decrease of Rubisco and chlorophyll content (Paul and Stitt, 1995; Paul and Driscoll, 1997). The decrease of N content in plant biomass at elevated CO2 compared to ambient CO2 is usually within the range of 10–15% (Figure 1; Jablonski et al., 2002; Kim et al., 2003; Martin et al., 2002; Malamy, 2005; Wingler et al., 2006; Gutierrez et al., 2007). This decrease could be due to a range of factors. First, it could involve a dilution of N in plant tissues by an increased flux of photosynthetic compounds from excess carbohydrate accumulation. Second, there could be decreased N uptake due to both source effects (soil-root specific) and reduced demand (down-regulation of photosynthetic enzymes). Fourth, a decreased N assimilation capacity could be involved. Finally, there could be a reduced electron flow for nitrate assimilation. In particular, the photosynthetic C reduction cycle and nitrate assimilation compete for electrons from photosynthesis, and since CO2 assimilation is favored, this results in a reduced
N influx (Bloom et al., 2002, 2010; McDonald et al., 2002; Luo et al., 2004; Taub and Wang, 2008).

The decrease in N content in plants grown under elevated CO2 aggravates N-limiting conditions in the following years, as plant leaves senescence and drops to the soil, thus increasing microbial immobilization of N. Due to a high C:N ratio, the availability of N to plants further declines as more N is fixed in soil microbes. Consequently, this leads to progressive N limitation in most agricultural cropping systems (Ball, 1997; Luo et al., 2004). This suggests that N supply needs to be matched with higher C assimilation under elevated CO2, requiring new N management strategies in agriculture. Legumes have the potential to respond maximally to higher CO2 due to their N-fixing capacity by overexpression of N-assimilation genes, such as transgenic wheat plants displaying enhanced N assimilation capacity by overexpression of the GSII (Habash et al., 2001). Overexpression of the GSII gene in maize resulted in an increase of 30% kernel number (Martin et al., 2006). The limited benefits of modifications of such genes could be ascribed to (i) lack of suitable combinations of promoters and regulatory elements for specific expression of the target genes that can match with a certain growth stage, plant tissue type, or environmental conditions, and (ii) limited sink capacity in crop plants to assimilate and utilize additional N for increased growth and yield. Additional C skeleton and C assimilation would be required to expand sink capacity where surplus N can be incorporated. Overexpression of genes for N uptake, transport, and assimilation would ensure increased availability of N content and amino acids utilized for photosynthetic machinery which mainly control plant growth and development. Simultaneously, elevated atmospheric CO2 would ensure adequate C supply for enhanced plant growth. Hence, orchestrating coordinated efforts for growing C3 plants with increased NUE and better C assimilation capacity under elevated CO2 would be an effective strategy for avoiding CO2 or photosynthesis acclimation, leading to higher growth rates, yield, and quality in cereals.

CONCLUDING REMARKS

Plant growth is typically stimulated at elevated CO2, but often decreases with time, due to relaxation of photosynthesis to a lower rate under exposure to elevated CO2 over longer periods. The sustained and maximal stimulation of growth at elevated CO2 requires acquisition of additional N to maximize increased C assimilation. Coordinated efforts for increasing photosynthetic efficiency, enhancing sink capacity, and improving N uptake would potentially increase grain yield under rising atmospheric CO2. A marginal increase in crop growth and yield has been reported in several FACE experiments. Nevertheless, improving NUE and N uptake in crop plants could partially avert the limitations of both photosynthetic acclimation and reduced grain quality under elevated CO2 levels. The major private plant biotechnology companies are attempting to develop improved NUE transgenic lines in their research and development strategies. This would be an effective method and would reinforce their strategy for improved grain quality under commonly accepted climate change scenarios. In the past 15 years (1996–2010), the accumulated global land area for transgenic crops exceeded one billion hectares grown by over 15 million farmers (James, 2010). The use of genetically engineered crops has increased farmer profits, reduced herbicide and pesticide usage, and reduced chemical impact on the environment, which has mainly been achieved through single gene modifications. However, the traits of improving NUE and enhanced crop response to elevated CO2 are more complex and would require stacking of multiple modified genes. Efficient management of ammonium and nitrate application could also facilitate benefits of increased yield and sustained grain quality under forecasted atmospheric CO2 elevation. However, careful manipulations of N-responsive genes provide the greatest global advantages, since additional nitrogenous fertilizers pose undesirable economic and environmental threats.

ACKNOWLEDGMENT

We would like to thank Carolyn Rothstein for critical reading of the draft manuscript.
REFERENCES
Ainsworth, E. A., Fromm, B. E., Bernacchi, C. J., Long, S. P., Rogers, A., and答
tance and photosynthetic rate and carboxylase. Crop Sci. 38, 1467–1475.
ply have a major influence on the response of photosynthesis, carbon metabolism, nitrogen metabolism and growth to elevated carbon dioxid
Keating, R., Nielsen, M., Thr


Kant et al. Yield increase under elevated CO2


Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received 02 April 2012; accepted 30 June 2012; published online: 19 July 2012.


This article was submitted to Frontiers in Plant Genetics and Genomics, a specialty of Frontiers in Plant Science.

Copyright © 2012 Kant, Seneweera, Rodin, Materne, Burch, Rothstein and Spangenberg. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.