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Physical, Molecular gene expression and Biochemical changes occurring in a plant during elevated atmospheric CO_2

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Abstract
Carbon dioxide being a harmful gas to mankind its rise in atmosphere affecting the plants in physical, molecular process that modulate photosynthetic gene expression and biochemical processes that changes in CO_2 concentration to the production of metabolite signal. Increasing temperature and elevated carbon dioxide were the main reason for change in the climatic factors that affect plant strength and flowering allied events. Reduction in ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) protein results from species dependent variation in differential use of molecular processes. Many studies indicates that hexokinase functioning as a hexose flux sensor that ultimately affecting transcription products were the key for repression in photosynthetic gene expression. Hexoses are produced as signals primarily by sucrose cycling and secondarily by starch hydrolysis. Finally, this review gives research knowledge over the changes in physical, molecular gene expression and biochemical processes during elevated carbon dioxide levels.

Keywords: Carbon dioxide, gene expression, Rubisco, photosynthesis

Introduction
Carbon dioxide in the recent years is becoming the most dangerous gas by warming the earth's surface to a greater extent. Its concentration increased from 300 ppm to 402 ppm within a century and "Todays rate of increase is more than 100 times faster than the increase that occurred when the last ice age ended" undoubtedly, the most important greenhouse gas in the atmosphere. Changes in land use pattern, land clearing, agriculture, and other activities has led to rise in the carbon dioxide emission. CO_2 is released naturally into the atmosphere through volcanic eruptions and animal respiration but it is also released through human activities such as deforestation and the burning of fossil fuels for energy. Most of the CO_2 emitted by fossil fuel combustion remains in the earth's atmosphere while the remains are absorbed by the natural land, ocean and reservoir. CO_2 also spends a long time in the atmosphere increasing its impact.

Table 1: Atmospheric carbon dioxide growth rate over a years.

Decade	Atmospheric CO_2 growth rate
2010-2019	2.40 ppm per year
2000-2009	1.97 ppm per year
1990-1999	1.50 ppm per year
1980-1989	1.61 ppm per year
1970-1979	1.28 ppm per year
1960-1969	0.85 ppm per year

Atmospheric CO_2 concentration has risen at an accelerating pace since the start of the Industrial Revolution. For the 1000 years prior to the Industrial Revolution, CO_2 was stable at about 270 ppm. Today CO_2 concentration in the atmosphere is approximately at 402 ppm and by the middle of this century it is predicted to reach 550 ppm and to surpass 700 ppm by the end of the century.
Since the industrial revolution, humans have increased atmospheric CO_2 concentration by 30%. Systematic monitoring of the atmospheric concentration of CO_2 dates back to 1958 and the creation of the monitoring program at the Mauna Loa Observatory in Hawaii. The current observations taken at the Mauna Loa Observatory at Hawaii ensures that 2018 will be the year

carbon dioxide officially cross the symbolic 413 ppm mark. Never to return below it in our lifetimes. The CO_2 we already committed to the atmosphere has warmed the world about 1.8° F since the start of the industrial revolution.

According to some interpretations, however, even the median emission scenario from the IPCC report suggests that we are in a trajectory leading toward a rapid quadrupling of the preindustrial CO_2 atmospheric concentration. Hence the present review was collected to see the physical, molecular and biochemical changes that occurs during elevated CO_2.

a. Physical changes of a plant during elevated CO_2.

What happens to the growth of forest trees under elevated CO_2 ?

Agro-ecosystems may be strongly influenced by the projected increase in atmospheric CO_2 concentration and associated climate change. The direct effect of elevated CO_2 concentration on plant growth is of particular interest because of the possibility of increasing crop yields in the future once the substrate of photosynthesis and gradient of concentration between atmosphere and leaf will increase. The most evident and best studied effect of elevated atmospheric CO_2 is the so called "fertilization effect".

If the increase in atmospheric CO_2 will be accompanied by an increase in air temperature, crops may shorten their growing cycle, which may offset the advantages of an increasing CO_2. Therefore interactive effects of CO_2 concentration and temperature on plant growth is complicated. How climate change will affect crop yield will be critical for Agriculture as an enterprise and food supply activity worldwide. Changes in flowering time with elevated CO_2 have implications at several levels of biological organization, including the species, community and ecosystem levels (Springer and Ward, 2007) [34]. At species level, changes in flowering time are likely to influence plant evolutionary processes, mainly because flowering time is an important life history trait that has large effects on reproductive output and potential fitness (Jagadish *et al*., 2016) [13]. Typically for annual species, the onset of reproduction marks the end of the vegetative growth stage and begins the early stages of senescence. As a result, elevated CO_2 may alter plant fitness both through changes in flowering time and through altered plant size at flowering, which influences the amount of resources available for reproduction (Springer and Ward, 2007) [34].

Growth and productivity

It is clear that photosynthesis is enhanced by elevated atmospheric CO_2 and that long-term down-regulation in photosynthesis may not occur, it is far less certain what will happen with long-term growth and productivity under elevated atmospheric CO_2. This uncertainty arises for several reasons. First, most studies with trees have been with small trees, for short duration, and inside greenhouse or field chambers that modify the environmental conditions and do not allow for interactions with other natural stressors. Secondly, it is becoming increasingly clear that interactions with other factors such as soil fertility atmospheric pollutants and soil moisture can offset the elevated atmospheric CO_2 ''fertilization effect'', when trees are exposed under more natural forest conditions. Thirdly, almost all studies of elevated greenhouse gases on forest trees have either doubled the gas concentration or done a single large addition; thus, very little is known about the dose response and interactive effects of varying doses of greenhouse gases. With elevated CO_2, for example, little is known about how plants and plant communities will respond to the addition of 50–150 ppm above ambient.

Above-ground growth and productivity

The average improvement of photosynthesis for trees exposed to elevated CO_2 has been about 60%. The enhanced photosynthesis has generally been followed by a similar, albeit a somewhat decreased magnitude, enhancement of above-ground growth. Growth enhancement for trees exposed to elevated CO_2 has been about 27% with responses again varying with species, soil fertility O_3 levels, and year (Norby *et al*., 1999) [28]. Whether or not the positive growth responses to elevated atmospheric CO_2 will be maintained through the life cycles of trees is not known. During the exponential growth phase, from planting to crown closure, trembling aspen (*Populus tremuloides* Michx.) and paper birch (*Betula papyrifera* Marsh.) growth enhancement under elevated atmospheric CO_2 has been maintained for 4 years. Growth enhancement of a 10-year old loblolly pine (*Pinus taeda* L.) forest by elevated CO_2 resulted in a few years of growth stimulation (DeLucia *et al*., 1999) [5]. However, this was followed by sharply decreased growth after the third year of exposure, most likely because soil fertility became a limiting factor.

From studies to date, we know that the life-long aboveground growth response of forest trees in forest stands cannot be accurately predicted from short-term greenhouse or chamber studies (Norby *et al*., 1999) [28] or from step increases in CO_2 concentrations of one age class of trees alone. Studies are needed under realistic forest conditions where trees are exposed to elevated CO_2 in competitive situations, under natural co-occurring stresses, and for the lifetime of the stand.

Below-ground growth and productivity

Root systems comprise up to half the total tree biomass and below-ground net primary production (NPP) may exceed 50% of total NPP. Because C allocation to roots is often favoured over C allocation to shoots in plants grown under elevated atmospheric CO_2, below-ground function of forest ecosystems may change significantly.

Increased root growth of forest trees under elevated atmospheric CO_2 has been reported by several researchers. Consistent findings show that the production and mortality of fine roots produced by trees growing under CO_2 enrichment are significantly increased. Species differ in the responsiveness of their root systems to increased atmospheric CO_2, suggesting that differences in the ability of certain species to compete against others could be dramatically changed under elevated CO_2. It is not clear what effect these increased rates of fine-root turnover will have on C storage in the soil (Pritchard *et al*., 2001) [31]. In addition, little is known about CO_2 effects on the growth, development, and Carbon storage capacity of large, structural roots.

Carbon sequestration

There is growing interest in the capacity of forest trees and forest ecosystems to sequester carbon. Carbon sequestration is a complex process that is difficult to measure as growth, yield, net primary production, and C turnover are often confused with C sequestration. Carbon sequestration by forests can be quantified on the basis of their net ecosystem productivity. This is net primary productivity after subtracting the heterotrophic respiration caused by decomposition of above- and below-ground litter. However some studies revealed that higher temperatures increased the soil

respiration when exposed to elevated CO_2. Therefore, increased soil C sequestration of trees growing in elevated atmospheric CO_2 has not yet been demonstrated.

Mineral cycling

Studies has revealed that Nitrogen level in litter has decreased at elevated CO_2 but, quantity of litter increases about 20–30% under elevated atmospheric CO_2. Although bulk of literature in this area suggests that decrease in litter N, coupled with an increase in lignin concentration, results in a slower decomposition rate (Norby *et al*., 1999) [28].

Water balance

Nearly 70% of water vapour produced from ecosystems passes through leaf stomata, continues to be a great attention in how elevated atmospheric CO_2 affects stomatal conductance and forest stand-level transpiration. Long-term findings of forest trees have shown a substantial 21% decrease in stomatal conductance with elevated CO_2. Because of enlarged size of trees under elevated CO_2, question remains: 'Do trees use more water or less water even if stomatal conductance is decreased. Similarly, there is ambiguity whether water use efficiency will really be upgraded in forest stands, as has been suggested from immediate water use efficiency estimates from small and isolated trees. Stand-level transpiration capacities for forest trees under elevated CO_2 have only been made on a few species (Wullschleger and Norby, 2001) [47] this remains an important research need.

Wood quality and chemical composition

Wood and pulp quality are known to be affected by factors such as wood density, early versus late wood amounts, juvenile wood, fiber length, branchiness, branch thickness, and wood chemical composition. Oren *et al*. (2001) [30] reported a decrease in specific gravity from 0.52 to 0.48 g cm^{-3} for loblolly pine being grown under elevated CO_2. The decrease was similar in magnitude to what they reported for the same trees under fertilization reported no changes in lignin content, fiber length.

Phenology

Elevated atmospheric CO_2 concentrations affect the phenology of bud break and bud set, flowering time, length of time to seed set, leaf senescence and drop, and branch and shoot development rates. The most thoroughly studied phenological events have been spring bud break and autumn bud set.

Elevated CO2 and individual plants

In general elevated CO_2 favours higher photosynthate (sugars and starch) accumulation in plants (Springer and Ward, 2007) [34]. Elevated CO_2 has generally shown to increase crop growth. Many researchers have reported that the effect of elevated CO_2 concentration on plant growth shows large intra-specific and inter-specific variations. Various researchers found enhanced numbers of specific parts *i.e.*, branches, tillers and flowers when treated with elevated CO_2.

Elevated CO2 responses at different developmental stages of plants

1. Disparity in seed germination and leaf emergence
2. Leaf carbon dynamics
3. Whole plant response
4. Differential species growth
5. Variation in seed yield
6. Changes in seed bank demographics

Impacts of elevated CO2 at physiological level

At high CO_2 concentrations, the net photosynthetic rate of leaf is accelerated due to increased substrate availability for Rubisco enzyme. Springer and Ward in 2007 [34] showed that wheat crop grown at elevated CO_2 had increased level of chlorophyll pigments. Generally, plants display increased growth at elevated CO_2 that is associated with lower transpiration and increased photosynthesis. This enhanced photosynthesis often increases total non-structural carbohydrate (sugars and starches) concentrations within leaf tissues. Recent studies indicate that carbohydrates function as hormone like signals in many important physiological and developmental processes, such as the expression of genes involved in the control of photosynthetic down regulation at elevated CO_2 and in the initiation of flowering (Jagadish *et al*., 2016) [13]. Elevated CO_2 causes an increase in the intercellular or sub-stomatal CO_2 and that reduces the aperture of the stomatal pore leading to partial stomatal closure. Doubling CO_2 concentration may reduce the conductance at leaf level by 30-40%.

Characterizing the response of stomata to elevated CO_2 is important for understanding the effect of elevated CO_2 on crop response. Stomata close in response to elevated CO_2. The mechanism behind stomata closure in response to elevated CO_2 concentration is not clear yet. Stomata apparently do not respond directly to the CO_2 concentration around the leaf. The CO_2 sensor for stomatal action is considered to be located in the epidermis and is presumably in the guard cells, the inner lateral walls of which are permeable to CO_2. Plants tend to regulate CO_2 concentration in the stomatal cavity (Ci), so that for a given vapour pressure deficit there is a constant ratio Ci with the atmospheric concentration, Ci/Ca. The ratio Ci/Ca in stationary conditions is about two-thirds for C3 and one-third for C4 plants. Thus, a Ci/Ca regulation would lead to the partial closure at elevated CO_2 concentration.

Studies have shown that short-term gain of elevated CO_2 may be offset in the long-term by a negative acclimation of photosynthetic capacity. One of the mechanisms of negative acclimation of photosynthetic capacity after long-term treatments with elevated CO_2 concentration is the decreased activity and content of Rubisco. Also, negative acclimation has been associated to an imbalance between source capacity and sink capacity. The rate of photosynthesis at the source might exceed the capacity of the sinks at elevated CO_2.

Impact of elevated CO2 on phenology

Phenology refers to the science of appearance and has been principally concerned with the dates of first occurrence of biological events in their annual cycle. CO_2 elevation and associated climate warming is expected to change seasonal biological phenomenon such as plant growth and flowering, which is driven by environmental factors. Elevated atmospheric CO_2 concentrations affect the phenology of bud break and bud set, flowering time, length of time to seed set, leaf senescence and drop, and branch and shoot development rates and have a wide range of consequences for ecological processes. Important climatic factors affecting crop phenology are:

1. Temperature
2. Atmospheric CO_2 levels
3. Precipitation

The expected increase in CO_2 may potentially affect the crop phenology in two different ways: It may cause an increase in the surface temperature of the crop, and higher photosynthetic rates that can be realized under elevated CO_2. Higher photosynthetic rates will likely occur because of a higher CO_2 gradient from the source to the chloroplast and a higher reduced carbon gradient from the leaves to the sink organs. This will cause faster filling of sinks and perhaps the occurrence of earlier leaf senescence.

The most thoroughly studied phenological events have been spring bud break and autumn bud set. Bud break is either delayed or advanced under elevated atmospheric CO_2. Increased frost injury and increased winter dieback have both been described for trees growing under elevated atmospheric CO_2 in northern regions. Others have described a possible increased cold hardiness for some trees growing under elevated atmospheric CO_2 due to the build-up of soluble sugars that may act as cryoprotectants. This variation in CO_2-induced phenology responses suggests that species differences play an important role and that additional study is needed to determine major trends in CO_2 effects on phenology.

Impacts of elevated CO2 on flowering time

Flowering time in 40 published studies involving both crops and other plant species exposed to elevated CO_2 (from 350 to1000 ppm) showed 28 cases in which flowering time was earlier (average 8.6 days) and 12 cases in which flowering was delayed (average 5.2 days) The effect of elevated CO_2 (680 ppm) on a grassland ecosystem using FACE facility in California resulted in forbs flowering 2–4days earlier, while in the dominant grass community flowering time was delayed by 2–6days (Springer and Ward, 2007) [34]. Reproductive traits in non-crop and wild species are known to respond less to elevated CO_2 than in crop species. Contrasting results have been documented showing no effect of elevated CO_2 on grass species in amini-FACE experiment and similarly with maple tree.

These differential responses could lead to changes in relative flowering times between species, thus affecting the ecosystem. However, "Phenological complementarity" at an ecosystem level is known to promote coexistence of multiple species (Jagadish *et al*., 2016) [13]. Multiple environmental changes may alter the flowering time differentially in a natural landscape but the same would be more limited due to the domestication and breeding for uniformity in phenology among the crop plants. In general, elevated CO_2 favors higher photosynthate (sugars and starch) accumulation in plants (Springer and Ward, 2007) [34]. A sugar signaling metabolite trehalose-6- phosphate (T6P) showed a strong correlation ($r2$ = 0.94) with vegetative and shoot-apical meristem tissue sucrose levels in *Arabidopsis* (Wahl *et al*., 2013) [43]. T6P has been proposed to relay data about tissue carbohydrate availability and act as key signal for floral induction (Jagadish *et al*., 2016) [13].

However, in *Arabidopsis thaliana,* the effect of elevated CO_2 on flowering time is the net result of positive effect of elevated CO_2 on growth and negative effect resulting from its tendency to increase leaf number, diluting the carbohydrate concentration in leaves at flowering (Johnston and Reekie, 2008) [15]. Interestingly, in *A. thaliana*, exposure to elevated CO_2 significantly showed progressive flowering time within a generation; however, across15 generations, elevated CO_2 did not advance flowering time to the same level in each succeeding generation (Teng *et al*., 2009) [37]. Although the research was conducted in controlled environments, it provides clues that plants grown at elevated CO_2 for short-term may not evolve specific adaptations to elevated CO_2. Besides variable responses across non-crop species, studies with agricultural crops have shown an overall positive impact of elevated CO_2 on growth and yield. Most of this positive effect was credited to a longer vegetative phase due to delays in flowering time under elevated CO_2, e.g., in pigeon pea (Sreeharsha *et al*., 2015) [35]. Flowering time is mainly sensitive to a variety of biotic and abiotic stresses that are expected to become more dominant under future climates. Crops (or specific cultivars) with more flexibility to adjust flowering time to ensure vulnerable development phases escape stress. Thus, molecular mechanisms regulating flowering time under a combination of elevated CO_2 with different biotic and abiotic stresses would help in tailoring climate resilient crops and utilize additional carbon for increasing yields under future climate.

Floral induction pathways

Vernalization pathway

Vernalization pathway, the plant maintains silenced, due to chromatin condensation, the genes that activate flowering, while at the same time turns on different floral repressors in a signal dependant on low temperatures. In this pathway the gene called Flowering Locus C (*FLC*) plays a pivotal role because it represses the expression of the floral integrator genes: flowering locus t (ft), leafy (lfy) and supressor of overexpression of Constans (SOC1).

Hormonal pathway

The hormonal pathway includes internal signals coming from different hormones in order to promote flowering and is probably involved in the crosstalk between reproduction and other developmental or stress processes. Gibberellins are particularly decisive because they can also directly modify the expression of the floral integrators, so that mutants in the metabolism of gibberellic acids or in the repressors of their regulatory network are late flowering.

Photoperiod pathway

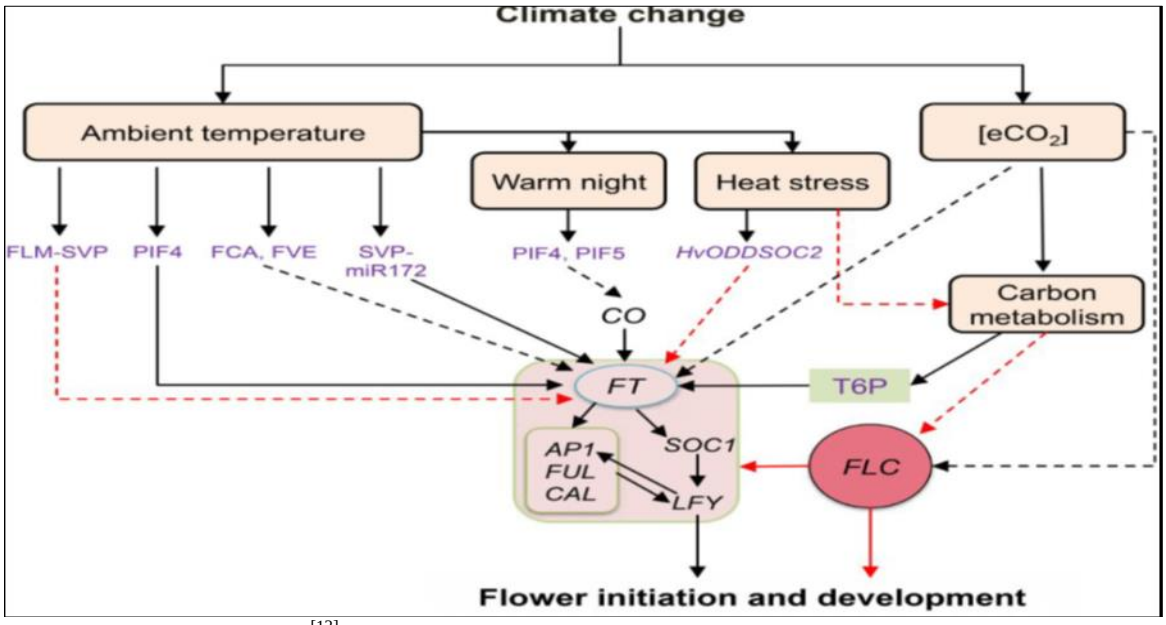
Probably the most important and most conserved of the flowering-time pathways is the photoperiod pathway that innerves signals coming from light quality and quantity to the ones coming from the circadian clock in order to promote flowering. Photoperiodic flowering depends on the gene *CONSTANS* so that mutations in this gene modify the response of the plant to day length. *CO* codifies for a protein exclusive to the plant kingdom with distinct amino acid domains that define a new class of transcription factors.

Impact of elevated CO2 on subsequent photoperiod pathway

Effect of elevated CO_2 on flowering time has been reviewed by Springer and Ward (2007) [34] who summarized 60 studies including 90 different crop and wild species grown under e[CO_2] in controlled chambers and the field (using open top chambers and FACE) conditions. The physiological basis of elevated CO_2 effect on flowering time was to enhance relative growth rates, increase plant size at flowering and raise tissue sugar status (Springer and Ward, 2007) [34]. Contrasting responses of flowering time to elevated CO_2 among short and long day plants suggested a possible interaction of the photoperiod pathway with elevated CO_2 to regulate floral signaling (Johnston and Reekie, 1994). In *Arabidopsis*, the sustained expression of the floral repressor gene *FLC* was

reported to be associated with delayed flowering in the genotype that was selected for high seed yield under elevated CO_2 (Springer and Ward, 2007) [34]. Recently, *MOTHER OF FT AND TFL1* (*MFT*), a homolog of *FT* and *TERMINAL FLOWER1* (*TFL1*), has been identified as a candidate gene influencing flowering time with elevated CO_2 (Ward *et al*., 2012). In comparison to the impact of temperature, Climate Change and Flowering Time current literature on the involvement of elevated CO_2 influencing the expression of flowering genes is limited. Only recently, the involvement of sugars in the regulation of flowering has been reported in *Arabidopsis*, where inadequate sugar levels in the vegetative tissue (leaf) and shoot apical meristem produce TREHALOSE-6-PHOSPHATE (T-6-P) as a proxy signal for floral transition and initiation under inductive environmental conditions (Wahl *et al*., 2013) [43]. Interestingly, *Arabidopsis* plants with mutation in the *TREHALOSE-6-PHOSPHATE SYNTHASE* gene (AT1G78580) failed to flower, showing the essential role of sugar signaling (*T-6-P*) in regulation of flowering time (Jagadish *et al*., 2016) [13].

Elevated CO_2 can induce floral transition with enhanced substrate supply through increased photosynthesis (Springer and Ward, 2007) [34]. For instance, elevated CO_2 delayed flowering in *Arabidopsis* plants with a 41 and 105% increase in foliar sucrose and starch content, respectively (Bae and Sicher, 2004) [1], indicating differential response to foliar sugars levels below and above threshold limits. Thus, varying sugar concentration under elevated CO_2 within/across species provides further investigation to find links between elevated CO_2 and flowering competency.



***Source*:** Jagadish, *et, al.*, (2016) [13].

Fig 1: Flowering regulation by ambient temperature and elevated CO_2

Abbreviations: *AP1*, *APETALA1*; *CO*, *CONSTANS*; *CAL*, *CAULIFLOWER; FLC*, *FLOWERINGLOCUS C*; *FLM*, *FLOWERING LOCUS M; SVP,SHORT VEGETATIVE PHASE;FT*, *FLOWERINGLOCUST*; *FUL*, *FRUITFULL; HVODDSOC2, AMADS-BOXß ORAL REPRESSOR; LFY*, *LEAFY*; *PIF4, PHYTOCHROME INTERACTING FACTOR4*; *SOC1*, *SUPRESSION OF OVEREXPRESSION OF CONSTANS1*; T6P, trehalose-6-phosphate

Ambient temperature regulates *FT* expression by different mechanisms including PIF4 dependent and independent pathways. Warmer night temperature could induce early morning flowering by accumulating PIF, PIF5 which regulate *CO* mediated *FT* expression. Heat stress may affect flowering events by hampering carbon metabolism and sugar signaling or by inducing floral repressor *HvODDSPC2*. Conversely, elevated CO_2 may directly regulate *FT* expression through floral repressor *FLC* or alternatively, positive impact of elevated CO elevated CO_2 on carbon metabolism and sugar signaling may induce flowering pathway genes by suppressing *FLC* expression. Major flowering pathways genes affected by temperature and elevated CO_2 are represented in the box in the centre.

Floral modifications under elevated CO2

Plant reproduction is highly vulnerable to global climate change components such as carbon dioxide concentration (CO_2), temperature (T), and ultraviolet-B (UV-B) radiation. Pollen produced by the flowers of these plants appeared shrivelled without apertures and with disturbed exine ornamentation even at elevated CO_2 conditions. Elevated CO_2 produced smaller flowers with shorter standard petal and staminal column lengths. Flowers so produced had less pollen with poor pollen germination and shorter tube lengths.

b. Molecular level changes at elevated CO_2

Photosynthetic gene responses

Certain leaf Rubisco genes were identified at elevated CO_2 where, some reported decreased state and some cases reported an increased enzyme activation. Carbonic anhydrase facilitates diffusion of CO_2 from intercellular air spaces (Edwards and Walker, 1983) [7] and level of mRNA and carbonic anhydrase activity reduced at elevated CO_2. At elevated CO_2 species that are having decreased photosynthetic activity resulted low levels of Rubisco protein by 40% while, leaf chlorophyll level also resulted declined nature. Furthermore, calvin cycle genes may also be differentially affected at elevated CO_2. For eg., in wheat flag leaves, transcripts for Rubisco subunits and phosphoglycerate kinase are particularly sensitive to elevated CO_2 but seduheptulose-1,7-bisphophatase and phopshoribokinase mRNAs are not (Nie *et al.*, 1995a) [27].

Rubisco control

Transcription, post transcription and translation events are involved in control of Rubisco content (Berry *et al.*, 1986, Deng and Gruissem 1987) [2, 6]. Some recent studies revealed that elevated CO_2 Rubisco protein expression is co-ordinated with *rbcS* and *rbcL* mRNA levels (Webber *et al.*, 1984) [45].

Table 2: Influence of long term growth at 1000µ L L^{-1} CO_2 on Rubisco protein and subunit levels of 16 species (Moore *et al.*, 1986).

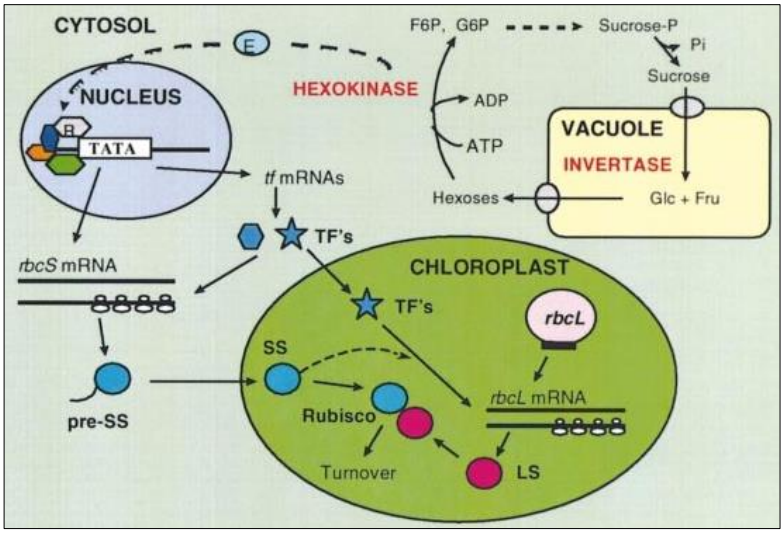
Species groups	% of ambient CO_2 content		
	Rubisco	rbcS mRNA	rbcL mRNA
A. Corn, parsley, pea, spinach	101	114	111
B. Cotton, sunflower, wheat, Bulge	84	61	115
C. Cucumber, soyabean, plantain	76	99	116
D-1. Bean, radish, tobacco, tomato	62	85	96
D-2. Arabidopsis	66	40	67

In group A Rubisco level was unchanged while, transcript levels increased at elevated CO_2. In group B Rubisco levels decreased, rbcS greatly decreased and rbcL reported no decline. In group C and D Rubisco content reduced to 25 to 40% at elevated CO_2 but, transcript levels reported no decrease with an exception of Arabidopsis where all Rubisco and transcript levels expressed a declined nature. Further tobacco decrease in Rubisco was found that it was not due to increase turnover of holoenzyme, but instead was associated with decreased synthesis of both subunits. However, at elevated CO_2 level the rbcL mRNA associated with polysomes was less than 40% in contrast to reduced change in rbcS, cab and psbA mRNAs. Rodemel *et al.*, (1996) reported that reduced association of rbcL mRNA with polysomes also occurred in transgenic tobacco containing rbcS antisense.

There has been considerable interest in determining whether sugar related may contain a response within their promoters. Sheen (1990) [14] found that sucrose repression of photosynthetic gene in a maize protoplast transient expression system was mediated by positively acting promoter elements upstream of TATA box. In another case study it was found that a 123 bp fragment of a malate synthase promoter was shown to mediate sugar repression in a transient cucumber protoplast expression (Graham, Baker and Leaver, 1994) [10]. The *cis*-element involved in sugar repression was further defined by 5¢ deletion analysis and found to be a 16 bp sequence, termed IMH2, in the malate synthase promoter. Maas, Schaal and Werr (1990) [21] reported that within the promoter of the maize Shrunken gene (*Sh1*, sucrose synthase), there occurs a 26 bp sequence responsive to sucrose repression. Recently, a sucrose repression element was identified in the *rbcS2* gene promoter (–203 to –187 bp) of *Phaseolus vulgaris* (Urwin and Jenkins 1997) [41].

Increased leaf carbohydrate content and decreased Rubisco protein content are common responses of plants exposed to elevated atmospheric CO_2. Krapp *et al.*, 1993 [18] examined the influence of added glucose and glucose analogs, in association with light CO_2 on rbcS transcript level. Treatment with glucose alone reduced rbcS mRNA level by 64% within 3 h. However, treatment with glucose analogs that are transported into cell did not repress the transcript levels. Moreover, in the absence of CO_2 but in presence of glucose, sugar levels were increased and amount of rbcS, minimally affected. These results suggest that hexose metabolism rather than sugar metabolism involved in sugar signalling that is related to Rubisco gene expression. There is also an evidence that carbohydrate signalling in plants can occur by alternate pathways independent of hexokinase (Mita *et al.*, 1997) [24]. In this sense hexokinase may function more as a response regulator than as a signal sensor. There is evidence that sucrose may function directly as signal metabolite. If hexokinase can function as flux sensor, then increase in cytosolic hexose level could result in increased carbohydrate flux to hexose-phosphate and thereby initiate the signal transduction response.

One alternative mechanism for increased provision of hexose as a sugar signals at elevated CO_2 could involve their daily mobilization from storage pools. Vacuolar hexoses may be mobilized around sunset, while chloroplastic starch may be degraded during sunlight.



***Source*:** Moore *et al.*, (1999).

Fig 2: A model describing molecular control of Rubisco protein content during photosynthetic acclimation to elevated CO_2. Molecular control involves many processes. As a result of transduction of carbohydrate signal produced. In tobacco these elevations include translation of rbcS and rbcL mRNAs, which we attribute to repression of specific translation factors (TF)

c. Biochemical level changes at elevated CO_2

A model was depicted describing the effect of elevated CO_2 on gene expression that is based on inter-relationship between sucrose cycling and carbohydrate signalling, and that is linked to previous molecular model. Sucrose cycling may produce leaf hexoses by either an intracellular or extracellular pathway. Vacuolar/Apoplastic hexoses produced by sucrose hydrolysis are transported to cytosol and ultimately phosphorylated by hexokinase. In this model, increased provision of hexoses from sucrose cycling increases carbohydrate signal by improved hexose flux through hexokinase. In Arabidopsis and wheat, diurnal fluctuations in rbcS transcripts and leaf carbohydrates occur in reciprocal patterns, with night time increase in rbcS mRNA associated with decrease in leaf sugars (Nie *et al.*, 1995a) [27]. This hypothesis proposes that circadian clock functions as a negative regulator of gene expression. We anticipate that CO_2 signal-response pathway must intersect at fundamental level with other processes that control plant growth and development.

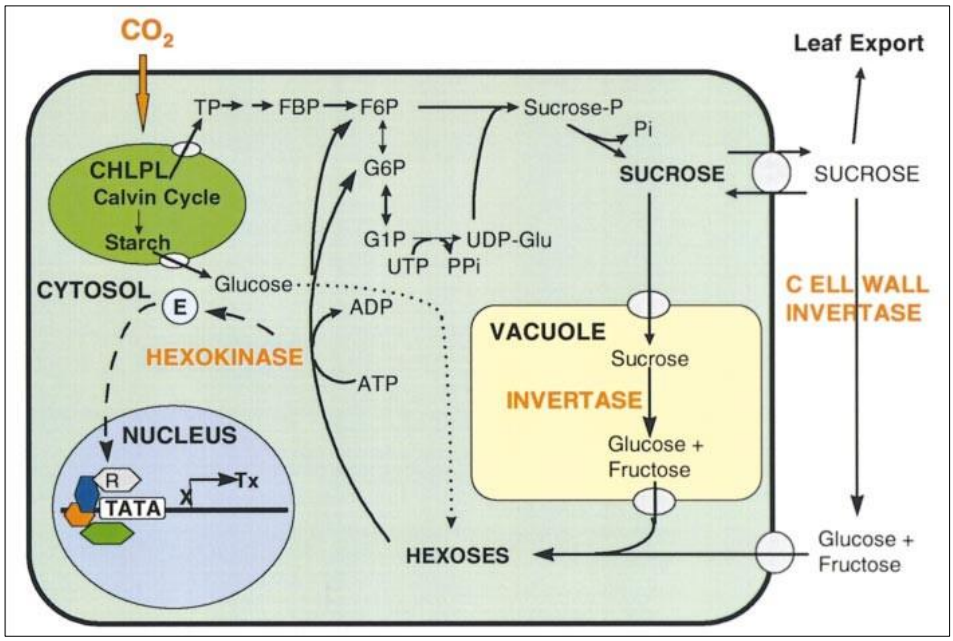


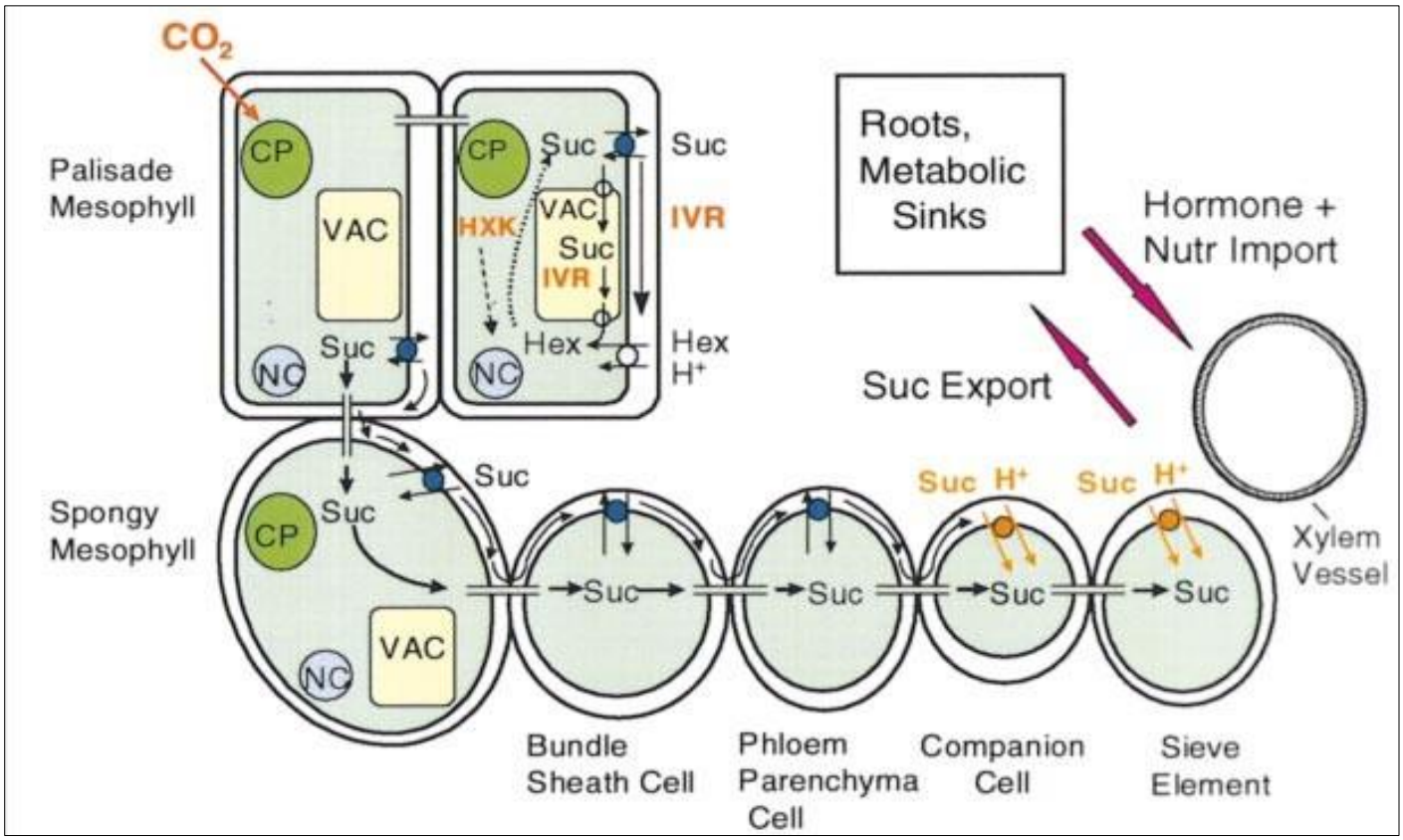
Fig 3: A model explaining leaf metabolism associated with production of carbohydrate as potential signal that affect photosynthetic gene expression at elevated CO_2. (Huber 1989; Jang and Sheen 1994 [14]; Moore *et al.*, 1998).

Sucrose is produced by cytosolic sucrose-phosphate synthase and phosphatase. Sucrose may then be maintained in cytosol, transported to the vacuole. Vacuolar sucrose can be hydrolysed by acid invertase. Resulting hexoses must be phosphorylated by hexokinase in order to re-enter cellular metabolism, thereby establishing a metabolic cycle. The primary carbohydrate signal would be generated by hexose flux through hexokinase as a result of sucrose cycling. A secondary pathway for hexose production may occur by chloroplastic starch hydrolysis. Sugar sensing of hexose flux by hexokinase may occur by modulation of protein effectors (E, which includes a kinase and phosphatase, Jang and Sheen 1997) that ultimately represses transcription of certain photosynthetic genes. There is also some evidence that carbohydrate signalling may occur by transport of external hexoses or sucrose across the plasma membrane, but it is not yet clear if the associated transduction processes affect photosynthetic gene expression. Notably, vacuolar invertase is thought not to be metabolically regulated *in vivo* other than by substrate availability (Kruger 1990) [19], but the activity of apoplastic invertase may be modulated by a protein inhibitor in a process perhaps subject to metabolic control by sucrose (Greiner, Krausgrill and Rausch 1998) [11]. See Fig. 2 for the molecular component of this model. TP, triose-phosphates.

In figure 4, a conceptual model was depicted that links the cell level model for carbohydrate signalling (Fig. 2) to relate components that affect leaf-level metabolism. It was suggested that relative limitation in leaf sucrose export and utilization may affect sucrose cycling within mesophyll cells. Increased leaf sucrose levels may produce by mass action effects an increased concentration of sucrose as substrate for vacuolar/apoplastic invertase, resulting in increased sucrose cycling and consequent photosynthetic down regulation. Plants are recognized to vary in the transfer of carbohydrates from mesophyll cells to sieve elements of the minor veins, using primarily either an apoplastic pathway or a symplastic pathway (van Bel 1993; Turgeon 1995) [19, 39]. Species that use symplastic transport may be less efficient in leaf carbohydrate export particularly at low temperatures (van Bel 1993) [19] and are reported to accumulate, on average, increased levels of starch at both ambient and elevated CO_2 in comparison with species that use apoplastic phloem loading (Körner *et al.*, 1995) [17]. However, when grown at least at moderate temperatures at elevated CO_2, such species may not acclimate to any different extent (Kingston-Smith *et al.*, 1998) [16]. Interestingly, some species, such as willow, may not concentrate sucrose in the phloem sieve cells (Turgeon and Medville 1998) [40]. Instead, sucrose may accumulate to unusually high levels in the mesophyll cells and directly diffuse to the phloem tissue.

While the majority of crop plants utilize an obligate apoplastic step in transfer of sucrose to phloem tissue (Giaquinta 1983; Fig. 4) [9], even the mechanism for the release of sucrose from mesophyll cells to the apoplast is not specifically known (van Bel 1993; Sauer *et al.* 1994) [19, 33]. Sucrose release may involve facilitated transport (Laloi *et al.*, 1993) [20] using a carrier analogous to ones thought to occur in certain sink cells (Patrick 1997), but the mesophyll carrier has not yet been identified at the molecular level (Ward *et al.* 1998). Sucrose is probably released to and then retrieved from

the apoplasm along the transfer paths, but this process has not been well characterized (Madore and Lucas 1989) [22]. The role of the apoplastic compartment in sucrose cycling and the response of apoplastic sugars to plant growth at elevated CO_2 should be also determined for species that vary in their degree of photosynthetic acclimation. Notably, after inhibiting phloem export by a cold-induced petiole girdle, leaf apoplastic sucrose levels rapidly and reversibly increased several-fold in *Vicia faba* (Nitska and Delrot 1986) [29]. In addition to possible signal effects (Fig. 3), leaf apoplastic sugars can affect carbon partitioning due to their increased solute potential affecting phloem turgor (Williams *et al.*, 1991) [46].



***Source*:** Moore *et al.*, 1999

Fig 4: A conceptual model depicting the leaf-level trafficking of sucrose, particularly as related to the generation of potential signals that affect mesophyll photosynthetic gene expression at elevated CO_2. (Modified from Chiou and Bush, 1996) [3]. Transfer of sucrose to the phloem tissue may occur, as shown, by either symplastic or apoplastic routes. Sucrose leakage to and retrieval from the apoplast is shown as possibly occurring along either transfer route, in a process that for simplicity is shown mediated by a single transporter. A relative limitation in the export of sucrose due to insufficient sink utilization of carbohydrate is suggested to result in increased storage in leaf cells and, by mass action effects, to result in increased provision of sucrose to vacuolar and/or apoplastic acid invertases. The integration of sink and source metabolism may occur by feed-forward effects from sucrose translocation to sinks (Farrar and Gunn 1996) and possibly by hormonal or other signals derived from sink tissues (e.g. Morris 1996). CP, chloroplasts; Hex, hexoses; HXK, hexokinase; IVR, invertase; NC, nucleus; Suc, sucrose; VAC, vacuole. Plasmodesmata are shown simply as single, intercellular bridges.

Elucidation of the control of assimilate partitioning by sucrose-mediated regulation of the sucrose symporter activity (Chiou & Bush 1998) [4] will improve our under- standing of leaf level responses to elevated CO_2, but is unlikely to explain all of the carbohydrate-related responses in part due to long-distance interactions between source and sink tissues (Fig. 4). Sucrose normally constitutes about one-half of the osmotic solutes in sieve elements. In one study, phloem sugar concentrations are reported from a C^{11}-tracer analysis to change diurnally and in response to different imposed changes in CO_2 concentrations (Magnuson *et al.*, 1986) [23]. However, short-term exposure to elevated CO_2 has little affect on the rate of leaf sugar export. This may occur because the rate of long-distance carbohydrate trans- port is a function of a phloem turgor pressure gradient between sources and sinks rather than being a function of phloem sugar concentrations.

Sink leaves have for long been recognized as preferentially utilizing for protein synthesis carbon derived from photosynthesis within the sink leaf (Turgeon 1989) [38]. Thus, increased protein synthesis in sink leaves due to increased photosynthesis may be an important component of plant responses to elevated CO_2. Coordination of sink and source activities at elevated CO_2 also may involve hormonal signals sent from source to sink tissues or from sink to source tissues. Additionally, such coordination will be affected by plant nitrogen metabolism (Stitt and Krapp 1999) [36]. Nitrate itself may function as a metabolic signal that can affect carbohydrate metabolism and acclimation to elevated CO_2. Understanding the nature and influence of long-distance signalling events on leaf-level responses to elevated CO_2 will be a challenge for future research efforts, of other environmental factors that influence photosynthetic acclimation to elevated CO_2.

Conclusion

From the present review it can be concluded that leaf carbohydrate content increased and Rubisco photosynthate protein decreased significantly during the high carbon dioxide

levels. Increased provision of hexoses from sucrose cycling increases carbohydrate signal by improved hexose flux through hexokinase. Thus, carbohydrate level increases greatly at elevated CO_2 levels.

References

1. Bae HH, Sicher R. Changes of soluble protein expression and leaf metabolite levels in *Arabidopsis thaliana* grown in elevated atmospheric carbon dioxide. Field Crops Res. 2004; 90:61-73.
2. Berry JO, Nikolau BJ, Carr JP, Klessig DF. Translational regulation of light-induced ribulose-1,5-bisphosphate carboxylase gene expression in amaranth. Molecular and Cellular Biology. 1986; 6:2347–2353.
3. Chiou T, Bush DR. Molecular cloning, immunochemical localization to the vacuole, and expression in transgenic yeast and tobacco of a putative sugar transporter from sugar beet. Plant Physiology. 1996; 110:511–520.
4. Chiou TJ, Bush DR. Sucrose is a signal molecule in assimilate partitioning. Proceedings of the National Academy of Sciences USA. 1998; 95:4784-4788.
5. DeLucia EH, Hamilton JG, Naidu SL, Thomas RB, Andrews JA, Finzi A. Net primary production of a forest ecosystem with experimental CO_2 enrichment. Science. 1999; 284:1177-1179.
6. Deng XW, Gruissem W. Control of plastid gene expression during development: The limited role of transcriptional regulation. Cell. 1987; 49:379–387.
7. Edwards G, Walker DA. C_3, C_4: Mechanisms, and Cellular and Environmental Regulation, of Photosynthesis. University of California Press, Berkeley, CA, 1983.
8. Farrar JF, Gunn S. Effects of temperature and atmospheric carbon dioxide on source-sink relations in the context of climate change. In Photo assimilate Distribution in Plants and Crops (eds E. Zamski & A.A. Schaffer), Marcel Dekker Inc, New York, 1996, 389-406.
9. Giaquinta RT. Phloem loading of sucrose. Annual Review of Plant Physiology. 1983; 34:347-387.
10. Graham IA, Baker CJ, Leaver CJ. Analysis of the cucumber malate synthase gene promoter by transient expression and gel retardation assays. The Plant Journal. 1994; 6:893-902.
11. Greiner S, Krausgrill S, Rausch T. Cloning of a tobacco apoplasmic invertase inhibitor. Proof of function of the recombinant protein and expression analysis during plant development. Plant Physiology. 1998; 116:733–742.
12. Huber SC. Biochemical mechanism for regulation of sucrose accumulation in leaves during photosynthesis. Plant Physiology. 1989; 91:656-662.
13. Jagadish SVK, Bahuguna RN, Djanaguiraman M, Gamuyao R, Prasad PVV, Craufurd PQ. Implications of high temperature and elevated CO_2 on flowering time in plants. Frontiers in plant sciences. 2016; 7(916):1-11.
14. Jang JC, Sheen J. Sugar sensing in higher plants. The Plant Cell. 1994; 6:1665-1679.
15. Johnston A, Reekie E. Regardless of whether rising atmospheric carbon dioxide levels increase air temperature, flowering phenology will be affected. Int. J Plant Sci. 2008; 169:1210–1218.
16. Kingston-Smith AH, Galtier N, Foyer CH. Soluble acid invertase activity in leaves is independent of species differences in leaf carbohydrates, diurnal sugar profiles, and paths of sugar loading. The New Phytologist. 1998; 139:283-292.
17. Körner CH, Pelaez-Riedl S, Van Bel AJE. CO_2 responsiveness of plants: a possible link to phloem loading. Plant Cell and Environment. 1995; 18:595–600.
18. Krapp A, Hofmann B, Schafer C, Stitt M. Regulation of the expression of rbcS and other photosynthetic genes by carbo- hydrates: a mechanism for the 'sink' regulation of photosynthesis. The Plant Journal. 1993; 3:817–828.
19. Kruger NJ. Carbohydrate synthesis and degradation. In Plant Physiology, Biochemistry and Molecular Biology (eds D.T. Ennis & D.M. Urpin), Longman, Harlow, UK, 1990, 59-76.
20. Laloi M, Delrot S, M'Batchi B. Characterization of sugar efflux from sugar beet leaf plasma membrane vesicles. Plant Physiology and Biochemistry. 1993; 31:731–741.
21. Maas C, Schaal S, Werr W. A feedback control element near the transcription start site of the maize shrunken gene determines promoter activity. EMBO Journal. 1990; 9:3447–3452.
22. Madore MA, Lucas WJ. Transport of photoassimilates between leaf cells. In Transport of Photoassimilates (eds D.A. Baker & J.A. Milburn), Longman Scientific & Technical, Harlow, Essex, 1989, 49-78.
23. Magnuson CE, Goeschl JD, Fares Y. Experimental tests of the Münch-Horwitz theory of phloem transport: effects of loading rates. Plant, Cell and Environment. 1986; 9:103–109.
24. Mita S, Murano N, Akaike M, Nakamura K. Mutants of Arabidopsis thaliana with pleiotropic effects on the expression of the gene for α-amylase and on the accumulation of anthocyanin that are inducible by sugars. The Plant Journal. 1997; 11:841–851.
25. Moore BD, Cheng SH, Sims D, Seemann JR. The biochemical and molecular basis for photosynthetic acclimation to elevated atmospheric CO_2. Plant, cell and environment. 1999; 22:567-582.
26. Moore BD, Cheng SH, Sims D, Seemann JR. Sucrose cycling, rubisco expression, and prediction of photosynthetic acclimation to elevated atmospheric CO_2. Plant, Cell and Environment. 1998; 21:905-915.
27. Nie GY, Hendrix DL, Webber AN, Kimball BA, Long SP. Increased accumulation of carbohydrates and decreased photosynthetic gene transcript levels in wheat grown at an elevated CO_2 concentration in the field. Plant Physiology. 1995a; 108:975–983.
28. Norby RJ, Wullschleger SD, Gunderson CA, Johnson DW, Ceulemans R. Tree responses to rising CO_2 in field experiments: implications for the future forest. Plant Cell and Environment. 1999; 22:683-714.
29. Ntsika G, Delrot S. Changes in apoplastic and intracellular leaf sugars induced by the blocking of export in *Vicia faba*. Physiologia Plantarum. 1986; 68:145–153.
30. Oren R, Ellsworth DS, Johnson KH, Phillips N, Ewers BE, Maier C. Soil fertility limits carbon sequestration by forest ecosystems in a CO_2- enriched atmosphere. Nature. 2001; 411:469-472.
31. Pritchard SG, Davis MA, Mitchell RJ, Prior SA, Boykin DL, Rogers HH. Root dynamics in an artificially constructed regenerating longleaf pine ecosystem are affected by atmospheric CO_2 enrichment. Environ Exp Bot. 2001; 46:55-69.
32. Rodermel S, Haley J, Jiang CZ, Tsai CH, Bogorad L. A mechanism for intergenomic integration: abundance of ribulose-1,5-bisphosphate carboxylase small-subunit protein influences the translation of the large-

subunit mRNA. Proceedings of the National Academy of Sciences USA. 1996; 93:3881–3885.
33. Sauer N, Baier K, Gahrtz M, Stadler R, Stolz J, Truernit E. Sugar transport across the plasma membranes of higher plants. Plant Molecular Biology. 1994; 26:1671-1679.
34. Springer CJ, Ward JK. Flowering time and elevated atmospheric CO_2. New Phytol. 2007; 176:243–255.
35. Sreeharsha RV, Sekhar KM, Reddy AR. Delayed flowering is associated with lack of photosynthetic, acclimation in Pigeon pea (*Cajanus cajan* L.) grown under elevated CO_2. Plant Sci. 2015; 231:82–93.
36. Stitt M, Krapp A. The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. Plant, Cell and Environment. 1999; 22:583–621.
37. Teng N, Jin B, Wang Q, Hao H, Ceulemans R. No detectable maternal effects of elevated CO_2 on Arabidopsis thaliana over 15 generations. PLoS one. 2009; 4:6035.
38. Turgeon R. The sink-source transition in leaves. Annual Review of Plant Physiology and Plant Molecular Biology. 1989; 40:119–138.
39. Turgeon R. The selection of raffinose family oligosaccharides as translocates in higher plants. In Carbon Partitioning and Source–Sink Interactions in Plants (eds M.A. Madore & W.J. Lucas), American Society of Plant Physiologists, Rockville, MD, 1995, 195–203.
40. Turgeon R, Medville R. The absence of phloem loading in willow leaves. Proceedings of the National Academy of Sciences USA. 1998; 95:12055–12060.
41. Urwin NAR, Jenkins GI. A sucrose repression element in the Phaseolus vulgaris rbcS2 gene promoter resembles elements responsible for sugar stimulation of plant and mammalian genes. Plant Molecular Biology. 1997; 35:929–942.
42. Van Bel AJE. Strategies of phloem loading. Annual Review of Plant Physiology and Plant Molecular Biology. 1993; 44:253–282.
43. Wahl V, Ponnu J, Schlereth A, Arrivault S, Langenecker T, Franke A. Regulation of flowering by trehalose-6-phosphate signaling in Arabidopsis thaliana. Science. 2013; 339:704–707.
44. Ward JM, Kühn C, Tegeder M, Frommer WB. Sucrose transport in higher plants. International Review of Cytology. 1998; 178:41–71.
45. Webber AN, Nie GY, Long SP. Acclimation of photosynthetic proteins to rising atmospheric CO_2. Photosynthesis Research. 1994; 39:413–425.
46. Williams JHH, Minchin PEH, Farrar JF. Carbon partitioning in split root systems of barley: the effect of osmotica. Journal of Experimental Botany. 1991; 42:453–460.
47. Wullschleger SD, Norby RJ. Sap velocity and canopy transpiration in a sweetgum stand exposed to free-air CO_2 enrichment (FACE). New Phytol. 2001; 150:489–498.